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Emissions of Quaternary Alkylammonium Compounds (QUAT-Fate)

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Summary

In this project, a method to quantify 20 quaternary ammonium compounds (QUATs) at ng/L level was established. A novel approach for the identification of specific transformation products has been developed, resulting in the description of a total of 23 photodegradation products and 35 biodegradation products (metabolites) exclusively associated with BAC-12 (Benzalkonium chloride with a dodecyl alkyl chain). Some of these were also detected in environmental samples, such as urban runoff samples.

The market survey revealed a wide variety of products containing QUATs not only in the area of human hygiene (Product type 1, PT1) but also in the area of roof and terrace cleaning products in 2021. – Quaternary ammonium surfactants contained in fabric softeners have different structures as discussed under the Biocidal Products Regulation (BPR, regulation 528/2012, EU, 2012). An update of the market survey revealed that in 2023 the composition of the products as found in do-it yourself-markets was updated and especially BACs were substituted for a large variety of products by nonanoic acid (pelargonsyre).

Urban water runoff had a high variety of QUAT concentrations. – The maximum found in a controlled catchment was in average 2 µg/L but went in single samples as high as 10 µg/L. – The high values are actually exceeding EC_{50} values (5.9-280 µg/L) for fish, thus during the 2 observation years at least one event could have led to a fish kill in the receiving retention pond. However, the mass flows were usually below 1 g for each rain event, except in one event in which a mass load of 4-10 g (depending on the individual compound) was observed. Urban runoff is considered to be one of the three major issues on QUATs.

Wastewater treatment plants have high inputs with µg/L concentrations of QUATs, most probably originating from PT1 applications (human hygiene). However, the concentrations vary more than other biocides with a similar application background (e.g., triclosan). However, the effluents of these plants contain 99% less than the influents. Most probably, the removal mechanism is through fast sorption followed by slow biodegradation. However, sludge contains high amounts (up to 144 000 µg/kg or 144 mg/kg) of QUATs which might be considered to be the second major issue on QUATs. Biodegradation leads to formation of metabolites which represents the third significant concern regarding QUATs.

In surface waters, QUATs were detected every once-in-a-while with concentrations raising up to 100 ng/L. When changing from classical monitoring to a process study, it turned out that, no QUATs were detected during dry weather in any surface water sample. High QUAT-concentrations in surface water can be detected during rain events, however. High concentrations or inputs generally cannot be linked to wastewater treatment plants or wastewater but rather to urban water runoff.

Dansk Sammenfatning

I dette projekt blev der etableret en metode til at kvantificere 20 kvarternære ammonium stoffer (QUATs) med en detektionsgrænse under 1 ng/L. Metoden indeholder benzalkoniumklorider, dialkyldimethylammonium klorider, tetraalkylammonumklorider og trimethylmonoalkyammoniumklorider. Derudover blev der udviklet en metode til at identificere udvalgte transformationsprodukter. Treogtyve fotonedbrydningsprodukter og tyve bionedbrydningsprodukter (metabolitter) af BAC-12 alene blev karakteriseret. Nogle af disse blev også detekteret i miljøprøver, såsom regnvandsafløbsprøver

Markedsundersøgelsen afslørede en lang række produkter indeholdende QUATs ikke kun inden for human hygiejne (PT1), men også inden for tag- og terrasserengøringsprodukter i 2021. Kvaternære ammoniumstoffer indeholdt i blødgøringsmidler har en anden struktur end dem, som er diskuteret under EU biocidforordningen (EU, 2012). En opdatering af markedsundersøgelsen afslørede, at sammensætningen af produkterne i 2023 blev opdateret, og især Benzalkoniumklorider (BACs) blev erstattet af en lang række produkter med pelargonsyre.

Regnvandsafløb havde en høj variation af QUAT-koncentrationer. I et kontrolleret opland blev i gennemsnit fundet 2 µg/L, men nogen enkelte prøver har haft koncentrationer op til 10 µg/L. De høje værdier overstiger EC_{50}-værdier (5,9-280 µg/L) for fisk, og mindst én hændelse kunne således, have ført til fiskedød i det modtagende regnvandsbassin. Massestrømmene var dog sædvanligvis under 1 g per regn hændelse, bortset fra den ene hændelse, der allerede er diskuteret, hvor der blev observeret en massebelastning på 4-10 g (afhængig af individuelt stof). QUATs i separate regnvand anses for at være et af de tre store problemer med QUATs.

Spildevandsrensningsanlæg har høje input (µg/L koncentrationer) af QUATs. Til gengæld indeholder afløbsvandet fra disse anlæg 99 % mindre end tilløbet. Sandsynligvis er fjernelsesmekanismen hurtig sorption efterfulgt af langsom biologisk nedbrydning. Til gengæld indeholder slam store mængder (op til 144 000 µg/kg eller 144 mg/kg) QUATs, hvilket kan anses for at være det andet store problem med QUATs.

Bionedbrydning fører til metabolitter, hvilket er det tredje store problem med QUATs.

I overfladevand optræder QUATs en gang imellem med koncentrationer tæt på 100 ng/L. Ved skift fra klassisk overvågning til procesundersøgelse viste det sig, at der ikke kunne påvises nogen QUATs i recipienter over detektionsgrænsen i tørvejr. Høje koncentrationer blev fundet under regn hændelser. Høje koncentrationer eller input kan generelt ikke knyttes til spildevandsrensningsanlæg eller spildevand, men snarere til afstrømning af separate regnvand.

1. Introduction

Quaternary ammonium compounds (QUATs) are surface-active, antimicrobial, high production volume chemicals with a broad range of applications from agriculture (Mulder et al., 2018) or industrial processes (Zhang et al., 2015) to consumer products, like fabric softeners (Porter, 1994), cleaning agents, disinfectants, preservatives (Center for Disease Control, 2008), and personal care products (Li & Brownawell, 2010) (structural formulas in Figure 1-1). QUATs are salts of positively charged polyatomic ions of the structure NR_4^+, R being an alkyl group or an aryl group (IUPAC, 2006). The alkyl moiety of the molecule is not fixed, but permutated according to the intended use of the compound. In reality congener mixtures are used in all applications with side chain lengths of 8-22. The following different technical mixtures are used (Figure 1-1; Johansson et al., 2012; Kaj et al., 2014):

(1) didecyldimethylammonium chloride (DDAC, Bardac 22™) with C8-C20 alkyl groups.
(2) tetradodecyl-ammonium chloride (TDAC),
(3) alkyldimethylbenzyl ammonium chlorides (alkyl benzyldimethylammonium chlorides, benzalkonium chlorides; ABDACs, BACs,), with C8-C18 alkyl groups, and
(4) alkyltrimethyl ammonium chlorides, with C10-C22 alkyl groups (ATMACs).

The quantity of QUATs sold in the Nordic Countries in 2010 has been estimated to total 5196 tons, the major congeners being alkyl-trimethyl ammonium chlorides (442 tons), benzalkonium chlorides (1218 tons), dialkyl-dimethylammonium chloride (2813 tons) and “others” (723 tons) (Kaj et al., 2014).

QUATs are used both as detergents/surfactants (to clean) (Madsen et al., 2001), as softeners (in washing agents) and as biocides (to kill/ or hinder growth of microorganisms) (Wieck et al., 2018; EU, 2012). QUATs have been shown to have antimicrobial activity (Jia et al., 2001), acting against bacteria (including cyanobacteria), fungi, amoeba, and enveloped viruses. Being cationic surfactant type active substances, QUATs interact with the cell walls of microorganisms and block their functioning. The main use as detergents are probably softeners and cleaning of buildings, including rooftops (Gromaire et al., 2015) as well as pesticide formulations (Mulder et al., 2018). Concerning their application as biocides, use in i) human hygiene (both at home laundries and in health care institutions (Lasek et al 2019)) ii) food production plants and iii) veterinary hygiene (Mulder et al., 2018) are probably using the highest amounts of these compounds. Additionally, smaller usages in other fields, such as wood protection, exist. Use as detergents and biocides underly different regulations (ECHA 2016), although both types of products reach the environment.

According to their use, the emissions of QUATs are diverse: i) Use in human hygiene and as softener result in loads in wastewater and emissions via treated wastewater and sewage sludge are possible (Martinez-Carballo et al., 2007a and 2007b). ii) Use in veterinary hygiene result in direct emission with manure to soil (Mulder et al 2018), while iii) those used on buildings may result in emissions with stormwater (rain runoff) (Van de Voorde, et al., 2012; Gromaire et al., 2015) and are discharged from the stormwater sewers, where separated sewer systems are installed.

QUATs are relatively toxic to non-target organisms such as fish, e.g., for benzalkonium chloride an EC_{50} for marine and freshwater-biota ranging between 5.9 and 280 µg/L is documented (Gromaire et al., 2015). Also, the toxicity to Daphnia is pronounced (EC_{50}=20 µg/L, Zhang et al., 2015). Thus, there is suspicion that uncontrolled release of QUATs has been involved in fish kills in Denmark in recent years (Dagens, 2014; Jyllandsposten, 2014, Danmarks Radio, 2020). QUATs have been demonstrated to cause resistance to common antibiotics (Hansen et al., 2007; Piddock, 2006). It is currently unknown how the different sources contribute to an environmental burden in Denmark.

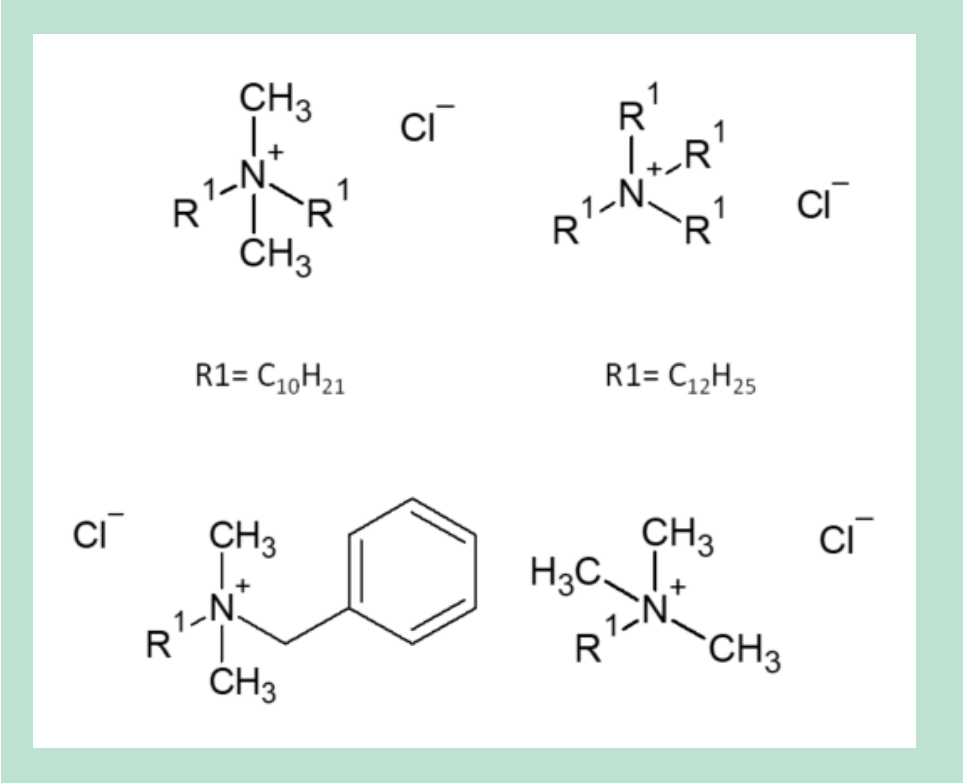


FIGURE 1.1. Examples of relevant QUATs congeners: (1) didecyldimethylammonium chloride (DDAC), (2) tetradodecyl-ammonium chloride (TDAC), (3) benzyldimethyldodecylammonium chloride, and (4) hexadecyltrimethylammonium chloride

Nomenclature:

Throughout the report the respective compounds will be referred to by their basic structure (see Figure 1-1), e.g., BAC for benzalkonium chloride followed by the number of the carbon atoms in the longest alkylic chain in the molecule. Thus BAC-14 refers to benzyldimethyl-tetradecylammonium chloride.

2. Hypothesis & Objectives

Hypothesises

The project was undertaken based on the following five hypotheses:

1. Transformation products of QUATs are relevant and their contribution to the mass balance/total emission need to be elucidated in real world systems.
2. Certain products containing QUATs that are not subject to the BPR (e.g., roof cleaning agents, softeners) contribute to the emissions of QUATs to surface waters, while simultaneously are neglected by the current exposure scenarios in the registration procedure of the BPR of products containing QUATs.

3a. It is possible to discriminate between biocidal and non-biocidal QUATs, using wastewater markers (e.g., benzotriazole), thus assessment whether WWTPs or surface runoff introduces most QUATs into the recipients.

3b. Discrimination between biocidal and non-biocidal use is possible.

4. Biotic and abiotic removal mechanism need more mechanistic understanding for reliable prediction of environmental fate of QUATs.

Objectives

The overall goal of this project was to experimentally determine the current emission for the quaternary ammonium compounds (QUATs) to the Danish aquatic ecosystems. The project's specific objectives were:

- To elucidate the fate of these compounds during application, in soil, and surface waters.
- To elucidate the fate of these compounds in wastewater treatment plants.
- To determine the quantity and identity of QUAT compounds sold in Denmark, by means of a market survey supported by chemical analysis.
- To quantify emissions of these compounds into surface waters of Denmark.

3. Materials and methods

3.1 Analytical methods

Blanks:

The analysis of QUATs has significant blank issues:

In analytical chemistry, blanks refer to samples or control solutions that do not contain the analyte of interest or any other substances being measured. Blanks are used to establish a baseline or reference point for comparison with other samples. They help identify and account for any background contamination or interference that may be present in the analytical method or instrumentation. By analysing blanks alongside the samples, analysts can differentiate between the measured signal resulting from the analyte and any background noise or contamination, ensuring accurate and reliable measurements. High blanks occur already in the standard HPLC instruments, as QUATs are obviously built into the flow path of the instruments. These can be overcome (to a vast part) by introducing a trap column between the pump and the autosampler, resulting in a system blank peak for the respective BAC appearing in the chromatogram after the peak originating from the injected sample (see Figure 3.1)

High blanks also were observed in the laboratory, as, especially during the COVID pandemic, QUATS were used as hand disinfectants to decrease transmission of SARS vira. - QUAT-containing disinfection agents were banned from the respective laboratory rooms and replaced by ethanol-only disinfectants. All glassware was burnt at 450°C.

All in all, it was decided to develop a method with minimal sample handling and blank samples were taken on field trips and during all laboratory handling of samples.

Quantitation:

All the surface water samples, and wastewater samples were analysed for 20 QUATs (BAC-6 to -18; DDAC-8 to -18; ATMAC-8- to -18; TDAC-6; TDAC-8 and TDAC-12 (compare Figure 1-1)) and for metoprolol which we used as marker for dilution of wastewater into the receiving waters. For the quantitation, separations by high performance liquid chromatography followed by detection by a triple mass spectrometer (HPLC-MS/MS) was applied. The chromatographic separation was performed by an HPLC system (1100 series, Agilent Technologies, Waldbron, Germany) equipped with a PFAS in line trapping column (Zorbax Eclipse, XDB-C18, 80Å, 5 µm, 4.6 x 50 mm, Agilent Technologies, USA) in the flow-path to remove compounds that are introduced as blanks from the HPLC system (such as PFAS and BACs). The mobile phases were: A) water and B) methanol each with 0.2% V/V formic acid (LiChrosolv, Darmstadt, Germany) added to each eluent. The separation column on the system was a reverse phase (Synergi Polar-RP, 4 µm particle size, 80 Å pore size, 150 x 2 mm, Phenomenex, Torrance, USA, equipped with a Synergi polar RP pre-column).

The QUATs were detected by means of a triple quadrupole mass spectrometer (API 5500, Sciex, Framingham, MA, USA), equipped with an electrospray ionization source used in positive mode. The MS/MS conditions are given in Table 3.1 while the HPLC conditions are given in Table 3.3. The total analysis time was 17 min and the injection volume was 10 µL. The data treatment was conducted with Analyst 1.6.3 (SCIEX, Framingham, USA). For quantification, two

different MRM (multiple reaction monitoring) transitions were used for each compound to obtain independent quantitative results. Only after checking for systematic deviations, the concentrations obtained from the two MRMs were averaged. In the rare case of deviating results from the two MRMS only the MRM which produced the lower numbers were finally processed, as it was assessed that false positives (if any), are more probable than false negatives. All quantitation was performed as internal standard calibration using deuterated QUATs as internal standards. Only for quality assurance studies, external calibration plus internal labelled standards were used to determine the linearity (R^2), the limit of detection (LOD) and the limit of quantification (LOQ) (Table 3.4). The LOD was determined as 3 times signal to noise ratio of the compounds signal obtained from blank methanol injections and the LOQ as 10 times signal to noise ratio from the same blanks. Calibration was conducted as 7-point calibration ranging from 0.1 to 100,000 ng/L. All samples were collected in triplicate and each triplicate was double injected. Example chromatograms are shown in Figure 3.1**.** The analytical sharp peak is clearly visible, e.g., for BAC 12 at 10.2 min, as well as the trapped system blank for BAC 12 at 10.5 min.

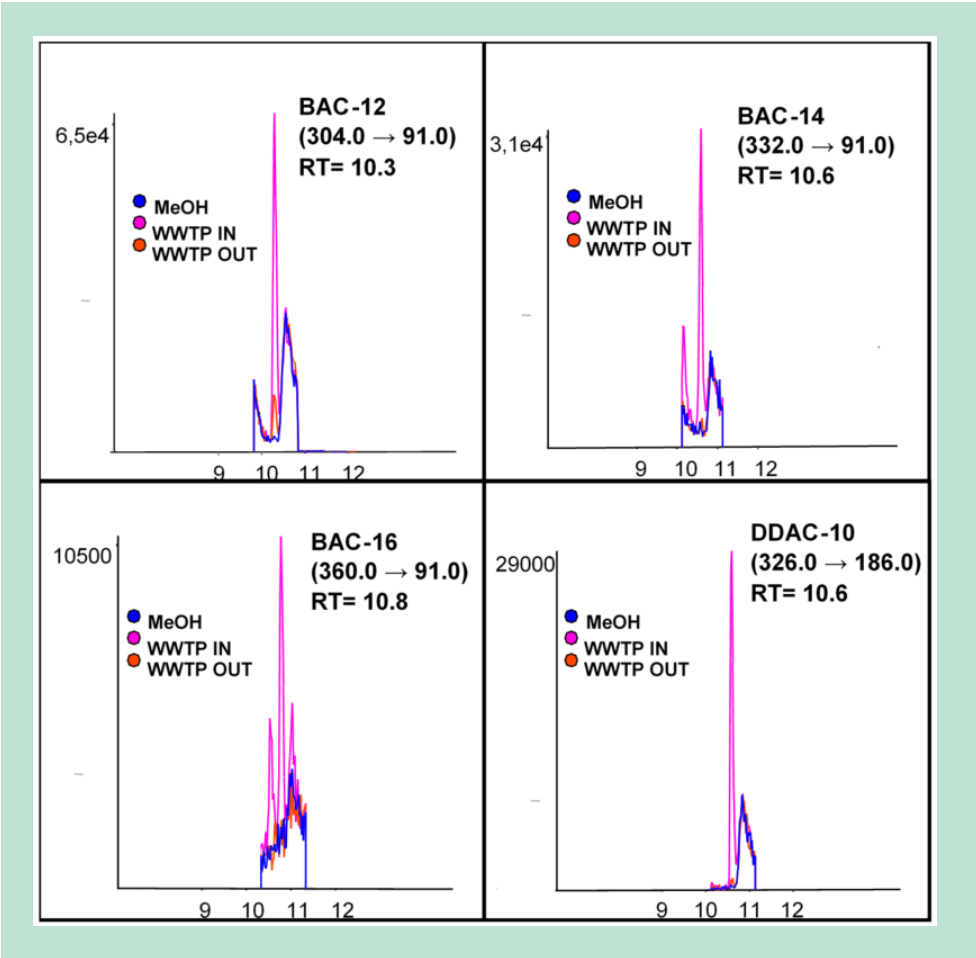


FIGURE 3.1 Extracted-ion chromatogram of DDAC-10, BAC-12, BAC-14 and BAC-16 as determined by HPLC-MS/MS in MRM mode

TABLE 3.1. Mass spectrometric condition for detection of QUATs

Compound	MRM	Retention time	Declustering potential	Collision energy	Collision cell exit potential
	[Da]	[min]	[V]	[V]	[V]
BAC-6	222.0 → 91.0	8.93	91	31	22
	222.0 → 128.0	8.93	91	23	22
BAC-8	248.0 → 91.0	9.45	91	38	22
	248.0 → 156.0	9.45	91	25	22
BAC-10	276.0 → 91.0	9.88	91	40	22
	276.0 → 184.0	9.88	91	26	22
BAC-12	304.0 → 91.0	9.80	91	47	22
	304.0 → 212.0	9.80	91	31	22
BAC-14	332.0 → 91.0	10.20	91	54	22
	332.0 → 240.0	10.20	91	31	22
BAC-16	360.0 → 91.0	10.40	91	59	22
	360.0 → 268.0	10.40	91	35	22
BAC-18	388.0 → 91.0	10.97	91	50	22
	388.0 →296.0	10.97	91	35	22
DDAC-8	270.5 → 158.0	10.04	91	35	22
	270.5 → 58.0	10.04	91	61	22
DDAC-10	326.0 → 186.0	10.20	91	40	22
	326.0 → 57.0	10.20	91	55	22
DDAC-12	382.0 → 214.0	10.93	91	42	22
	382.0 → 58.0	10.93	91	65	22
DDAC-14	438.5 → 242.0	11.17	91	50	22
	438.5 → 58.0	11.17	91	70	22
DDAC-18	550.5 → 298.0	11.57	91	55	22
	550.5 → 58.0	11.57	91	75	22
ATMAC-8	172.5 → 60.0	8.68	91	32	22
	172.5 → 71.0	8.68	91	31	22
ATMAC-12	228.5 → 60.0	9.71	91	42	22
	228.5 → 85.0	9.71	91	33	22
ATMAC-14	256.5 → 60.0	10.14	91	45	22
	256.5 → 85.0	10.14	91	33	22
ATMAC-16	284.5 → 60.0	10.49	91	48	22
	284.5 → 85.0	10.49	91	36	22
ATMAC-18	312.5 → 60.0	10.74	91	50	22
	312.5 → 85.0	10.74	91	38	22
TDAC-6	354.0 →128.0	10.67	91	49	22
	354.0 → 198.0	10.67	91	42	22
TDAC-8	466.0 → 354.5	11.18	91	50	22
	466.0 → 254.5	11.18	91	47	22
TDAC-12	691.0 → 523	11.80	91	68	22
	691.0 → 366.5	11.80	91	70	22

Metoprolol	268.0 → 116.0	5.87	91	23	50
	268.0 → 291.0	5.87	81	25	22
Benzotriazole	120.0 → 65.0	8.12	91	28	22
	120.0 → 92.0	8.12	91	25	22

TABLE 3.2. Mass spectrometric condition for detection of QUATs (Internal standards)

BAC-12-D7	331.5 → 98.0	9.80	91	47	14
	331.5 → 212.0	9.80	91	31	14
BAC-14-D7	339.5 → 98.0	10.20	91	54	10
	339.5 → 240.0	10.20	91	31	17
BAC-16-D7	367.5 → 98.0	10.40	91	29	17
	367.5 → 268.0	10.40	91	35	20
DDAC-10-D6	332.0 → 192.0	10.20	91	40	12
	332.0 → 57.0	10.20	91	55	10

TABLE 3.3. Flow- and gradient program of the HPLC for determining QUATs. (Eluent A: water with 0.2% formic acid, Eluent B: methanol with 0.2% formic acid)

Total time [min]	Flow rate [mL/min]	A [%]	B [%]
0.00	0.350	100.0	0.0
2.00	0.350	35.0	65.0
6.50	0.350	10.0	90.0
7.00	0.450	0.0	100.0
11.0	0.450	0.0	100.0
12.0	0.450	100.0	0.0
17.0	0.350	100.0	0.0

TABLE 3.4. Calibration equation, limit of quantification (LOQ), Blank and Correlation coefficient of the calibration data. Data from the biodegradation experiment.

Compound	R^2 1 st / 2nd Transition	Equation T1	Equation T2	LOQ (ng/L)	Blank (ng/L)*
BAC-6	0.9917 / 0.9973	y= 0.134x + 3.84	y= 0.0498 + 0.693	10.0	<0.1
BAC-8	0.9879 / 0.9663	y= 0.12x + 0.824	y= 0.0524x + 3.1	10.0	<0.1
BAC-10	0.9964 / 0.9909	y= 0.107x + 0.999	y= 0.0985 + 0.805	3.0	<0.1
BAC-12	0.9907 / 0.9940	y= 0.019x + 0.897	y= 0.109x + 0.813	1.0	<0.1
BAC-14	0.9911 / 0.9906	y= 0.0938 + 0.337	y= 0.0564x + 0.397	3.0	<0.3
BAC-16	0.6626 / 0.9919	y= 0.112 x + 0.669	y= 0.115x + 0.398	1.0	<0.1
BAC-18	0.9913 / 0.9965	y= 0.0742x + 0.115	y= 0.0765 + 0.871	10.0	<0.1
DDAC-8	0.9971 / 0.9948	y= 0.0602x + 0.0741	y= 0.0298 + 0.0352	3.0	<0.1
DDAC-10	0.9902 / 0.7898	y= 0.0277x + 4.1	y= 0.000234x + 0.00123	1.0	<0.1
DDAC-12	0.9916 / 0.9967	y= 0.0422x + 0.0422	y= 0.014x + 0.00641	10.0	<0.1
DDAC-14	0.9701 / 0.9907	y= 0.0452x + 1.55	y= 0.0151x + 0.0924	3.0	<0.1
DDAC-18	0.9954 / 0.9898	y= 0.009846x + 0.411	y= 0.00169x + 0.0652	10.0	<0.1
ATMAC-8	0.9953 / 0.9979	y= 0.0271x + 2.91	y= 0.00383x + 0.274	30.0	<0.1
ATMAC-12	0.9967 / 0.9914	y= 0.0244x + 0.175	y= 0.0349x + 0.212	30.0	<0.1
ATMAC-14	0.9978 / 0.9948	y= 0.0264x + 0.465	y= 0.00737x + 0.0595	30.0	<0.1
ATMAC-16	0.9941 / 0.9882	y= 0.00873x + 0.103	y= 0.00275x + 0.0907	30.0	<0.1
ATMAC-18	0.9871 / 0.9689	y= 0.0204x + 0.134	y= 0.0059x + 0.147	30.0	<0.1
TDAC-6	0.9941 / 0.1753	y= 0.0818x + 0.842	y= 0.00205x + 11.4	3.0	<0.1
TDAC-8	0.9920 / 0.9689	y= 0.073x + 0.0	y= 0.0059x + 0.147	1.0	<0.1
TDAC-12	0.9954 / 0.9964	y= 0.0148x + 0.0334	y= 0.0612x + 0.657	1.0	<0.1
Metoprolol	0.9947 / 0.9898	y= 0.24x + 0.000648	y= 0.333x + 0.00958	50	<0.1
Benzotriazole	0.9877 / 0.9851	y= 30.9x + 3.78	y= 18.8x + 0.232	100	<0.1

*due to the trap column the LC was able to separate the QUATs originating from the samples from the QUATs originating from the LC equipment.

3.2 Photooxidation products

To obtain access to photooxidation products, the single compounds were dissolved and diluted in HPLC-MS grade water to receive concentrations of 100 µg/L. These solutions were placed in glass vials, with a volume of 10 mL each, and subjected to UV light exposure. Samples were taken out after predefined time intervals. Exposure was conducted with and without humic acid as “natural” quencher.

3.3 Metabolites

Before start of the experiments, the carriers were incubated in an aerated glass reactor that was fed continuously (100 µL/min) with synthetic wastewater, i.e., an aqueous solution of 1 g/L ammonium bicarbonate, 10 g/L glucose, 1 g/L yeast extract and 0.1 g/L cellulose in tap water to allow biofilm development on the carriers. The carriers were in occulated with 5 ml activated sludge from a WWTP.

Degradation/metabolization was studied by means of incubations in micro MBBRs Larsson et al., 2023), which were conducted in triplicate. For these incubations carriers with biofilm grown and conditioned in 1 L reactors in synthetic wastewater were used in 100 mL Erlenmeyer flasks. The Erlenmeyer flasks contained 60 mL of tap water to maintain a similar environment for the biomass and four of the carriers with already grown biomass were added. No nutrients were added so the bacteria would use the BACs as main carbon source. The flasks were covered by aluminum foil to protect against light (prevent photodegradation) and potential dust contamination and put on an orbital shaker at 120 rpm.

The time series experiment was conducted as a batch experiment. The flasks were spiked with 1 mg/L BAC-12. To determine biodegradation rate constant and metabolite formation 1 mL samples were collected from these incubations over two weeks at time points 4, 8, 24, 30, 54, 78 144, 216, 288, 360, 456 and 552 hours.

The sample preparation was minimal as steps such as extraction and up-concentration can result in loss of metabolites with unknown structures. Therefore 1 mL sample was collected and put in a 2 mL HPLC autosampler vial, centrifuged and stored in -20°C before injection into a high-performance liquid chromatography coupled to high resolution tandem mass spectrometry (HPLC-HR MS/MS (see Chapter 3.4)).

3.4 Mass spectrometry for determining photooxidation products and metabolites

Metabolic analysis was done by SCIEX ExionLC 2.0+. UHPLC system (AB SCIEX LLC. Framinham. MA. USA) coupled to an API 6600 quadrupole time-of-flight (Q-TOF) mass analyzer (AB SCIEX. Framinham. MA. USA). The chromatic separation was performed with a reverse-phase column, i.e., an Acquity UPLC BEH Shield RP18 1.7 µm (2.1x 30 mm; Waters, Milford, MA, USA) column at 30°C utilizing injection volumes of 10 µL. The HPLC gradient consisted of two mobile phases: phase A was water with 0.1% formic acid and phase B methanol with 0.1% formic acid from Riedel-de Haën, Seelze, Germany). The flow was set to 0.3 mL/min and the gradient setup was: (1) 0–1 min. 0% B; (2) 1–9 min. 80% B; (3) 9–11 min. 80% B; (4) 11-16 min.

100% B; (5) 16-16.10 min. 0% B. The HRMS was operated with electrospray ionization (ESI) in positive (ESI+) mode. The MS parameters were: TOF mass range, 101-1200 Da; accumulation time, 250 ms; Ion spray voltage, 5.3 V; ion source temperature 500°C; collision energy spread of 20 to 50 eV. The HRMS was using information dependent acquisition (IDA) to generate product ion spectra during which TOF scan is used to trigger fragmentation of up to 10 ions per cycle (699 ms). To target the parent BAC-12 compound, a standard was injected to obtain retention time (RT), peak formation and protonated mass [M]+.

3.5 Description and selection of stormwater catchment

The stormwater catchment is a traditional Danish residential area containing single-family detached houses constructed in the 1970ies. It holds 140 single family homes in the outskirts of Silkeborg and covers 21.5 ha. The drainage area also comprises internal roads and part of an orbital road around Silkeborg. Its contributing area is 5.7 ha. The water flows to an underground monitoring station where samples were taken by an automated water sampler holding glass bottles. The drainage area is free of misconnected wastewater. From the monitoring station, the water runs to a treatment pond and from there to a receiving water body.

FIGURE 3.2. Aerial picture of the stormwater catchment

3.6 Description and selection of Wastewater treatment plants (WWTPs)

Samples from the influent and effluent were acquired as 24 h flow proportional composite samples from the Måløv, Stenløse and Ølstykke WWTPs (Zealand, Denmark; Figure 3.3)**.** The samples were taken on 9th and 10th July 2022 – this period did not experience any rainfall during the sampling period nor in the 48 h preceding the sampling. The characteristic of the three comparatively small WWTPs used for this balancing study, are shown in Table 3.5, together with the somewhat larger WWTP Bjergmarken (Roskilde, Denmark) from where the activated sludge samples for the biodegradation and sorption experiments were taken.

From these in- and outflow samples, concentrations, removal and partitioning to primary sludge were calculated.

FIGURE 3.3. Aerial view of the 3 WWTPs of Måløv, Stenløse and Ølstykke along the Værebro Å river course.

TABLE 3.5. Characterization of wastewater treatment plants. PE: person equivalent, COD: chemical oxy-gen demand, BOD: biological oxygen demand; CAS: conventional activated sludge, Fe: phos-phate precipitation with iron salts, Al: phosphate precipitation with aluminium salts, HRT: Hy-draulic residence time, SRT: sludge residence time.

	Måløv	Stenløse	Ølstykke	Bjergmarken
Size in PE	65000	16000	18000	125000
COD loading [PE]	43000	1200	15000	90000
Hydraulic flow [m3/a]	3716067	840557	1383361	6500000
Pre-clarifier	Yes	no	no	no
BOD removal	CAS	CAS	CAS	CAS
Nitrification/denitrification	CAS	CAS	CAS	CAS
P removal	Fe	Al + Biological	Al + Biological	Biological
HRT	43 h	63 h	40 h	24 h
SRT	25 days	25 days	25 days	25 days

3.6.1 Partitioning and removal of QUATs in WWTPs

The fate of QUATs in WWTP could be led by two different mechanisms: the sorption of the compounds to the sludge or by biodegradation during the hydraulic retention time in the WWTP.

3.6.1.1 Removal

To determine the removal, we used equation 1:

$$Removal\ [\%] = \frac{C_{influent} - C_{effluent}}{C_{influent}} * 100 \qquad \text{eq (1)}$$

$C_{influent}$ = Concentration of QUATs in the influent of the WWTP

$C_{effluent}$ = Concentration of QUATs in the effluent of the WWTP

3.6.1.2 Determination of partitioning to sludge (sorption constants)

Primary sludge

The sorption experiments concerning primary sludge were conducted following Ternes et al., 2004; Wick et al., 2011 *in situ*, without spiking. In detail: 15 mL of the influents of the WWTPs

were added to the respective glass screw cap vial and centrifuged for 10 min. 1 mL of the supernatant was taken and put into a 1.5 mL autosampler vial to determine the dissolved fraction. The remaining supernatant was removed carefully in order to leave the primary sludge (about 1 mL, approximately 200 mg dry weight) in the bottom of the glass screw cap vial. This 1 mL sludge was extracted in the same glass screw cap vial with a two-step extraction with 7.5 mL of methanol with 5 % of formic acid each, after adding internal standard solution, by manual resuspension followed by contacting in an ultrasonic bath for 30 min at 21°C. Successive to separation in the centrifuge, the methanolic supernatants were removed by means of Pasteur pipettes from the centrifugation vials.

All the samples were made in triplicates and were stored at 4 °C until analysis.

To determine the partitioning constants K_D we used the equation (2) (VanLoon and Duffy, 2011):

$$K_D = \frac{[Cs]}{[Cw]} \qquad \text{eq (2)}$$

[Cs] = concentration of QUATs in primary sludge or sludge

[Cw] = concentration of QUATs in water

If K_D is higher than 1 this means that the concentrations of the compound in the sludge are higher than in the water.

From the partitioning constants an organic matter based partitioning (K_{OC}) can be derived using eq (3) with foc = fraction of organic material in the sample (Schwarzenbach and Westall, 1981).

$$K_{oc} = \frac{K_D}{f_{oc}} \qquad \text{eq (3)}$$

Schwarzenbach and Westall (1981) derived equation (4) to relate K_{OC} to the octanol water partitioning constant KOW in soil related systems.

$$logK_{ow} = \frac{logK_{oc} - 049}{0.72} \qquad \text{eq (4)}$$

With log = decadic logarithm.

3.6.1.3 Sludge contacting and extraction for partitioning experiment

The sludge partitioning experiments were conducted with sludge that was taken in one batch from Bjergmarken WWTP, Roskilde. To assess partitioning of QUATs to sludge, 40 mL of sludge was utilised for each partitioning experiment. The experiments were conducted in glass screw cap vials (Corning Life Science, Mexico). To block degradation of QUATs during the partitioning experiments, 0.2%-v/w sodium azide (Sigma Aldrich, Darmstadt, Germany) was added. Partitioning experiments were conducted in triplicates for seven different QUAT concentrations to a) exclude or minimise effects of blanks and b) resolve concentration dependencies of partitioning. The concentration series was conducted with amended concentrations from 3 µg/L to 1 000 µg/L. The glass vials were put on a shaker table at 120 rpm at 21°C to for homogenizing and contacting. Samples were taken after 1 hour and again after 4 hours to assess the respective concentrations in solids and in the supernatants and thus the sorption equilibria. To separate solids from the aqueous phase, each vial was centrifuged for 10 min at 3000 rpm (1100 rotational force) on a Hermle Z 206 A centrifuge (Ole Dich Instrumentmakers, Denmark). Successively 1 mL of the supernatant was put into a 1.5 mL autosampler vial and internal standard solution was added. To measure the concentration of QUATs in the sludge, the supernatant was removed from the 40 mL contacting vial. About 4 mL of sludge were left in each glass vial. These were extracted with 36 mL of methanol with 5 % formic acid (Lichrosolv, purity: 98-100%, Merck,

Darmstadt, Germany). This mixture was resuspended and the QUATs were extracted from the solids by manual shaking and successively put on a shaker table at 120 rpm for 20 hours. Successively all the vials were put in an ultrasonic bath for 30 min and the methanolic phase was successively collected as supernatant after centrifugation. Internal standards were added to the extracts.

3.7 Biodegradation experiments

To assess the biodegradation of the QUATs in secondary sludge, 8 L of secondary sludge were collected from Roskilde (Bjergmarken) WWTP. The sludge was divided in four bioreactors (Figure 3.4). Two of them were used as control, two were spiked with a QUATs standard solution in amount to obtain a nominal contamination of 100 µg/L for each compound (for the real concentration see Table 3.6). Two bioreactors containing tap water were spiked with QUATs as positive control.

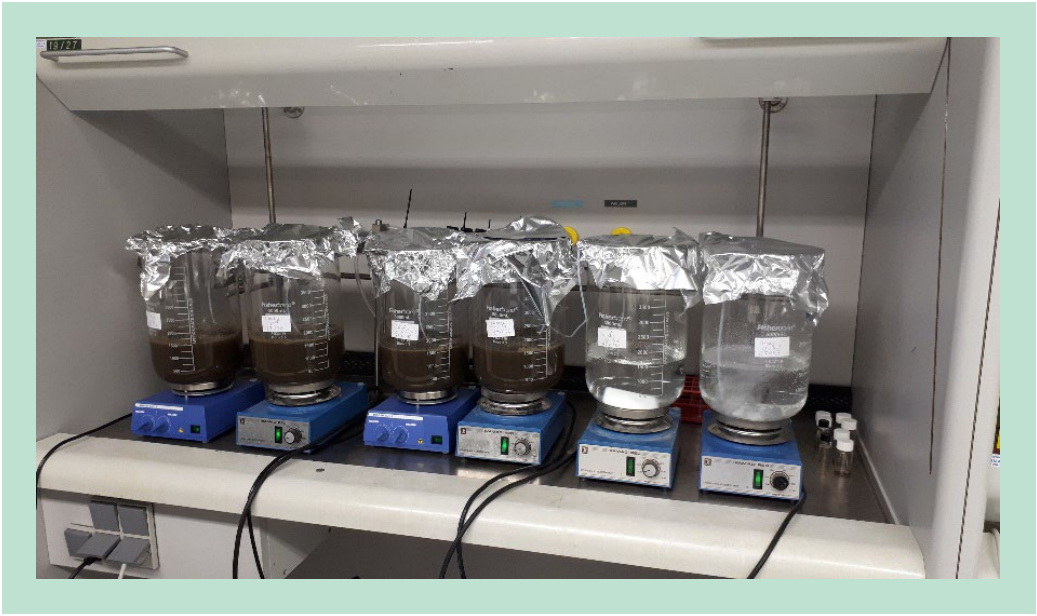


FIGURE 3.4. Set up for biodegradation experiments.

TABLE 3.6. Concentration of QUATs spike in the bioreactors

QUATs	Spike µg/L
TDAC-C8	81.80
ATMAC-C12	75.73
BAC-C6	72.33
ATMAC-C18	79.73
ATMAC-C14	109.40
ATMAC-C8	68.67
ATMAC-C16	66.67
BAC-C8	76.07
TDAC-C6	64.27
BAC-C10	68.40

BAC-C16	73.53
BAC-C14	71.73
BAC-C12	89.47
TDAC-C12	88.00
DDAC-C10	110.00
DDAC-C12	74.60
DDAC-C8	69.67
DDAC-C14	69.27
DDAC-C18	78.87
BAC-C18	68.40

The sampling procedure is show in Figure 3.5. In brief, at each time point, 10 ml of sample was taken over a period from 0 to 212 h.
The water and the sludge were separated by centrifugation and analyzed separately.
144 samples in total were collected (36 for each of the bioreactors).

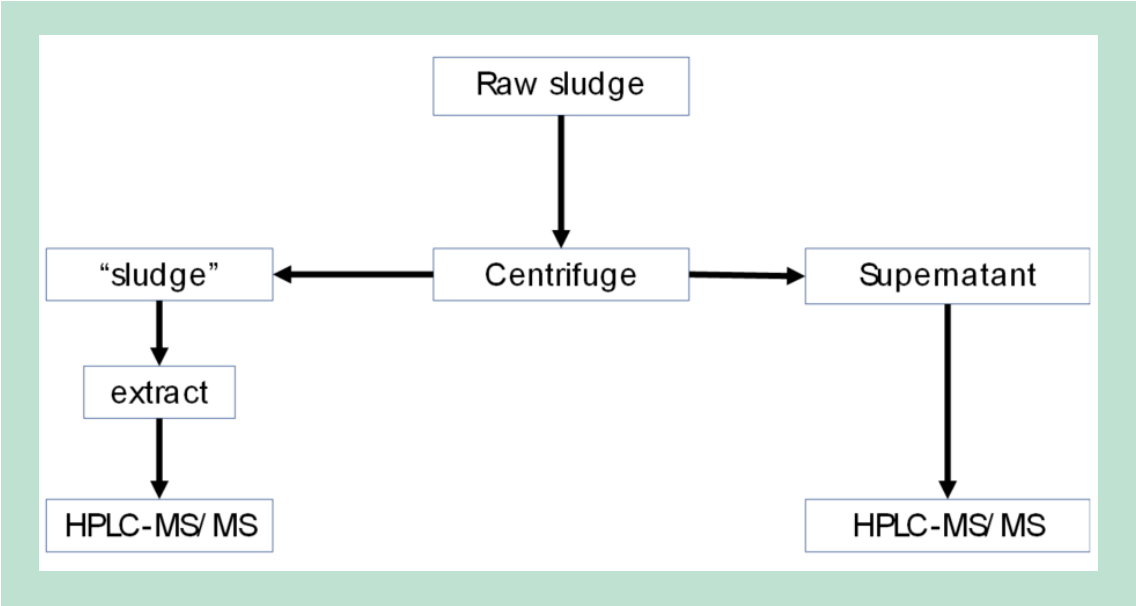


FIGURE 3.5. Sampling scheme during the biodegradation experiments.

Sludge extraction for the biodegradation experiment

For the extraction of QUATs from the biodegradation experiment. 10 ml was taken from the bioreactors and were placed into glass screw cap vials and centrifuged immediately. To measure the concentration of QUATs in the sludge, the supernatant was removed from the 10 ml contacting vial. About 1 mL of sludge were left in each glass vial and 100 µl of QUATs internal standard was added. These were extracted 2 times with 5 ml of methanol with 5 % formic acid (Lichrosolv, purity: 98-100%, Merck, Darmstadt, Germany). The mixture was resuspended by manual shaking and successively put in an ultrasonic bath for 30 min and the methanolic phase was collected as supernatant after centrifugation. Each vial was centrifuged for 10 min at 3000 rpm (1100 rotational force) on a Hermle Z 206 A centrifuge (Ole Dich Instrumentmakers, Denmark). Successively the supernatant was condensed to 1 ml by a Buchi syncore evaporator (BÜCHI Labortechnik GmbH, Germany) and transfered into a 1.5 ml autosample vial.

3.8 Selection and description of surface water catchment

QUATs emission have a broad usage in daily human activities as well as in agriculture, especially industrial husbandry, thus the selected rivers should be affected by cities, agricultural areas and have input from WWTPs. We chose to monitor the biggest river of Denmark (Gudenå, Jutland) affected by 7 WWTPs and from the 2 main cities of Silkeborg and Randers, a river of the Capital region of Denmark (Værebro Å) with several suspected sources of QUATs and one minor stream not affected by any WWTPs (Havelse Å, Zealand) (Figure 3.6). The sampling on Gudenå and Havelse Å rivers were done under dry weather and four samplings were done on Værebro Å river in dry and wet condition.

Surface water was collected in grab samples in duplicate at different locations from the river source to the mouth. For each sampling location, a duplicate sample of 1,5 L of surface water was collected in a glass bottle and refrigerated at 4 °C until analysis, which was performed max 3 days after the sampling (an aliquot of 10 ml for each sample was kept in the freezer for possible future analyses).
The sampling locations of the Værebro river (Figure 3.7) and the sampling stations of the other rivers are present in Table 3.7. Værebro Å, with a course 35 Km long, receives effluent wastewater from 3 different WWTPs, and flows through agricultural and rural areas between Måløv, Stenløse and Ølstykke. Several sampling campaigns were performed at different time points and seasons in 2021 and 2022 to investigate how different weather conditions affect the QUAT emissions.

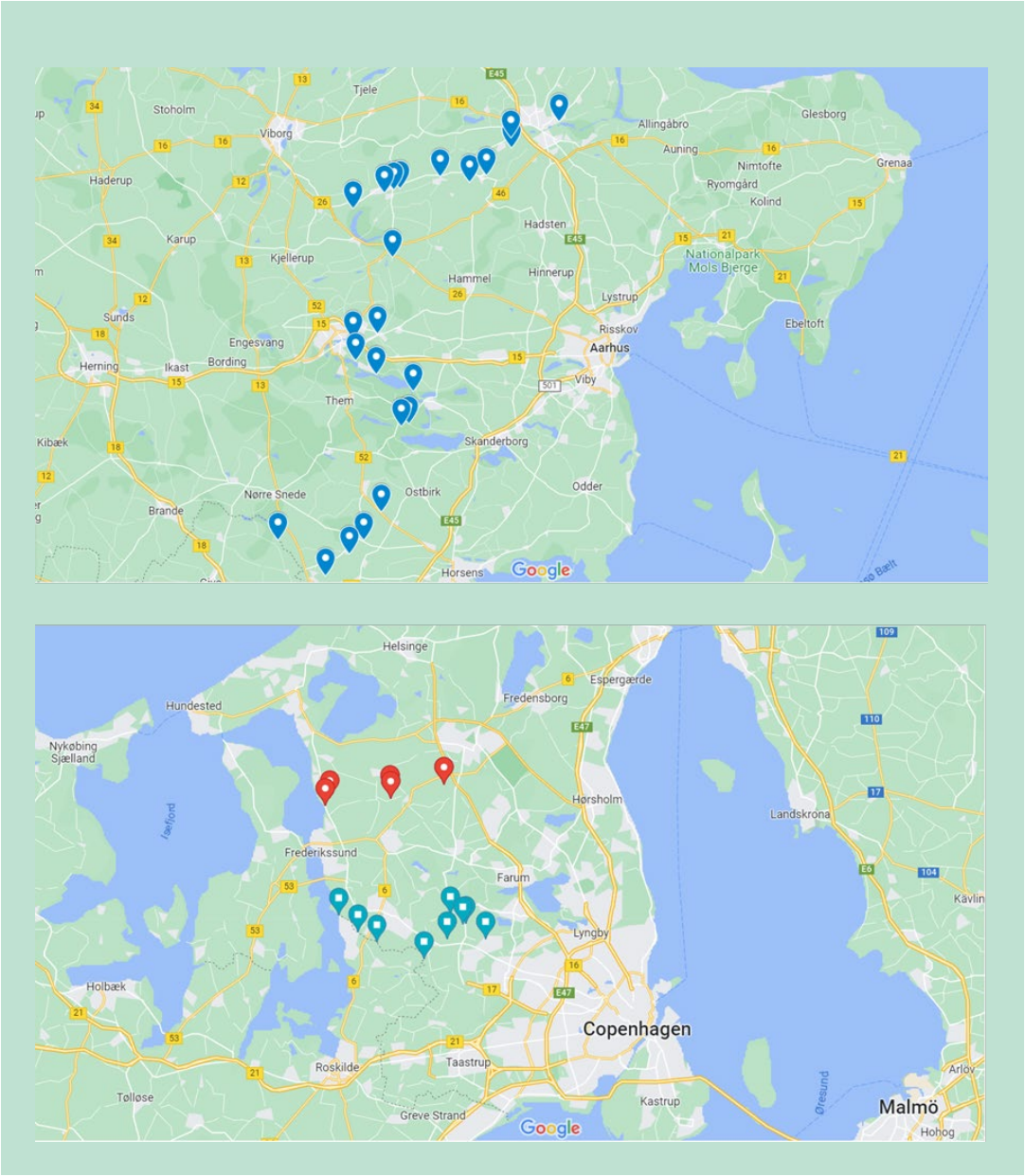


FIGURE 3.6. Map of surface water catchment (Gudenå (top) and Værebro Å and Havelse Å (below))

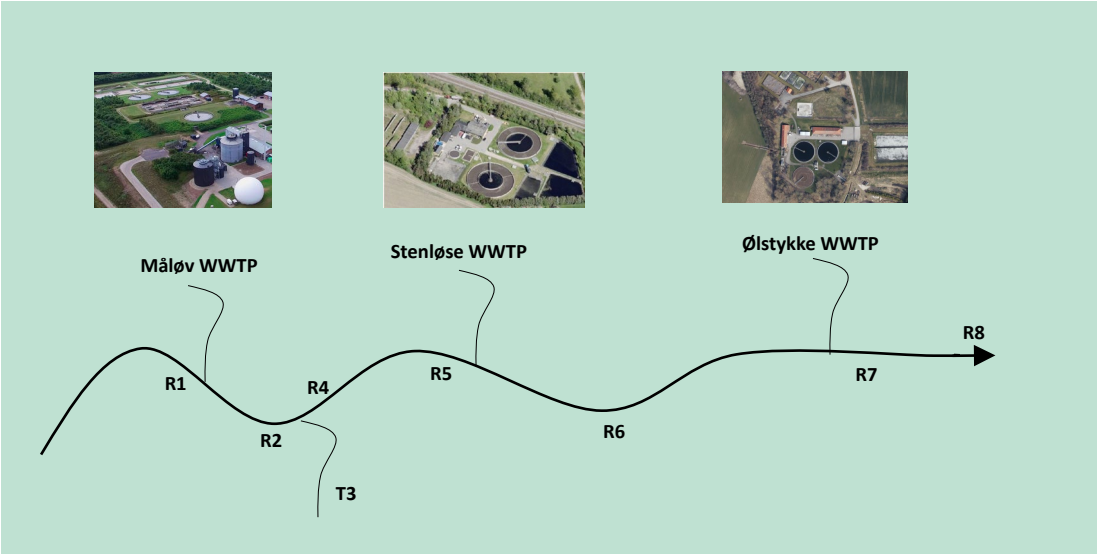


FIGURE 3.7. Sampling locations for a process study over the course of the river Værebro Å from Måløv town (location 1 upstream WWTP 1) to the mouth in Roskilde fjord (location 8).

TABLE 3.7. Number and description of the sampling stations along Værebro Å.

Station No	Værebro Å	Coordinatates
1	Upstream Måløv WWTP	55.758815288385385, 12.32567747823696
2	Downstream Måløv WWTP	55.772759510774605, 12.293453459710255
3	Tributary Bunds Å	55.77333883946129, 12.294107918693925
4	Værebro Å after Bunds Å tibutary	55.7720293030207, 12.28867912754614
5	Upstream Stenløse WWTP	55.73893533457683, 12.233146672429548
6	Downstream Stenløse WWTP	55.75525238817855, 12.152115573984213
7	Downstream Ølstykke WWTP	55.75991753224073, 12.133651247068737
8	Værebro Å mouth	55.779200, 12.091647

4. Method development

Instrumental blanks:

Instrumental blanks turned out to be crucial considering the QUATs. Several HPLC-MS/MS instruments were tested. Each instrument was tested several times with different washing/cleaning procedures:
Several discussions with other leading experts in the field revealed, that those that tried, had given up analysing QUATs due to high instrumental blanks. No one has up to now resolved whether the contaminating parts are the tubes, the degassers, the pumps or the separation columns.

1. API 4000 with a Dionex HPLC resulted in good calibrations but varying high blank values.
2. API 4000 with a Dionex HPLC with a Synergi trapping column resulted in good calibrations but varying high blank values.
3. API 5500 with a new Agilent HPLC resulted in good calibrations but high blank values.
4. API 5500 with an old Agilent HPLC with a Zorbax trapping column resulted in good calibrations and low blank values.

The API 5500 with an old Agilent HPLC with the Zorbax trapping column was chosen for this project.

SPE preconcentration:
SPE extraction with different formats were tested (DVB disks), Oasis HLB cartridges. Though extractions worked fine at high concentrations, at low concentrations blank values ruined the method performance and hoped for improved detection limits.

Final Method:
Thus, in the end direct injections on the BAC free HPLC-MS/MS system were conducted for quantitation as method of choice for the aqueous samples and minimal ultrasound supported extraction was conducted for the sludge samples.
Reliable quantitation and low blanks < 0.1 ng/L could be achieved (See Table 3-3).

5. Transformation products (WP1)

Work on transformation products was conducted to enable screening for these transformation products in the environment. However, the first step was to tentatively identify and characterise these. Considering use of the QUATs on surfaces like roofs and terraces (terrasse and roof cleaners), especially photodegradation is relevant. Considering the use of QUATs in human hygiene especially in product types (PT) 1 (human hygiene), PT 2 (disinfectants) and PT 4 (food and feed), biodegradation processes in wastewater treatment plants are relevant.

5.1 Phototransformation products

Phototransformation experiments were conducted in aqueous solutions as comparison between a) negative control (no QUAT), b) exposure and c) exposure with humic acid to get potential "natural" quenching and activation (Figure 5.1). The photodegradation experiments were conducted for BAC-12, ATMAC-12, DDAC-10, TDAC-8x, each for 48 h.

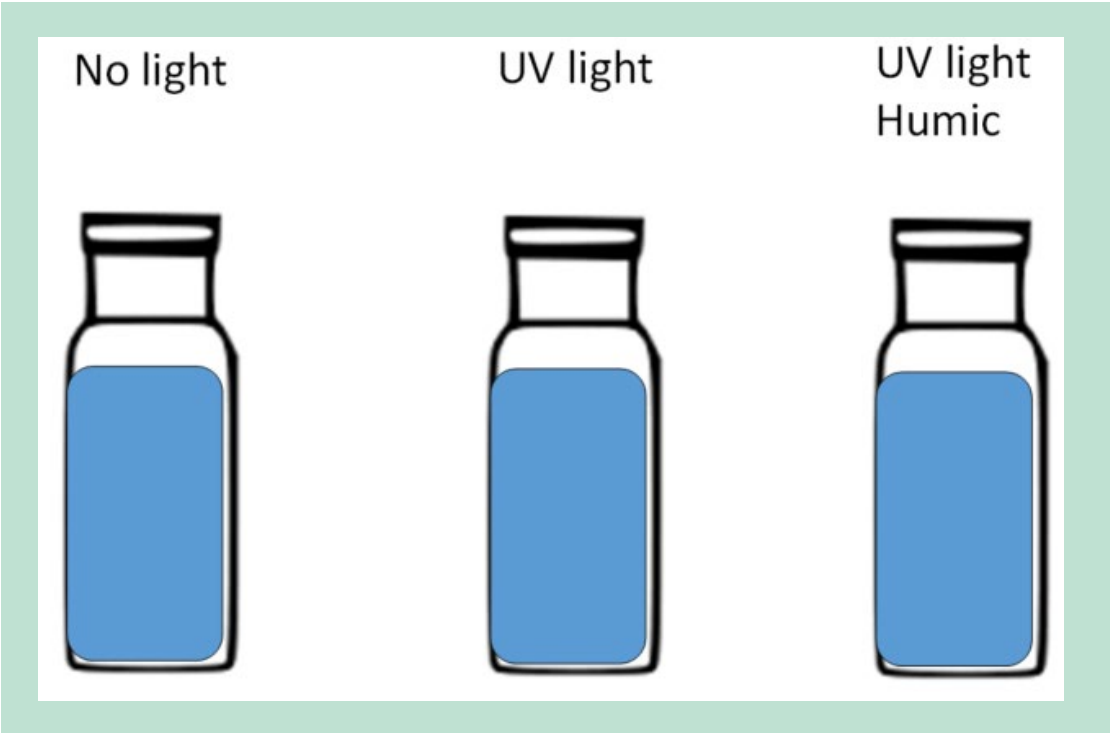


FIGURE 5.1. Photodegradation approach with exposure over 48 h.

After exposure, the samples were analysed by UHPLC coupled to high resolution mass spectrometry (Q-TOF).

Nomination

All photodegradation products will be nominated after their respective parent followed by PTP to indicate phototransformation products succeeded by their respective nominal mass. E.g., BAC-12 PTP320 is a phototransformation product with nominal mass 320 Da.
As expected, QUATs do photodegrade as shown in Figure 5-2 as example. Several reactions and products were observed:

5.1.1 BAC-12+O (BAC-12 -PTP320)

Photooxidation products (e.g., BAC-12 plus O) can be detected. In the following this will be called BAC-12+O or BAC-12 PTP320, following its nominal mass. This points towards the typical reactions of an organic molecule with OH radicals in which hydrogen atoms are replaced by OH-groups. At least 13 isomers of this transformation product can be resolved in different chromatographic peaks (in Figure 5.2). This would imply that photooxidation will occur on most carbon atoms of the molecule without much preference. From 6 of these isomers, it was possible to obtain mass spectra as shown in Figure 5.3.
Interestingly, the formation of the primary oxidation product is massively enhanced by the presence of humic acid. Obviously, humic acids are capturing the light and transferring the radicals to the BAC. For this process the humic acids transfer the energy either a) directly with a succeeding stabilisation reaction with water or by b) the activated humic acids produce OH radicals, which on their side react with the BAC. This process is faster than the direct photolysis of the BAC itself.

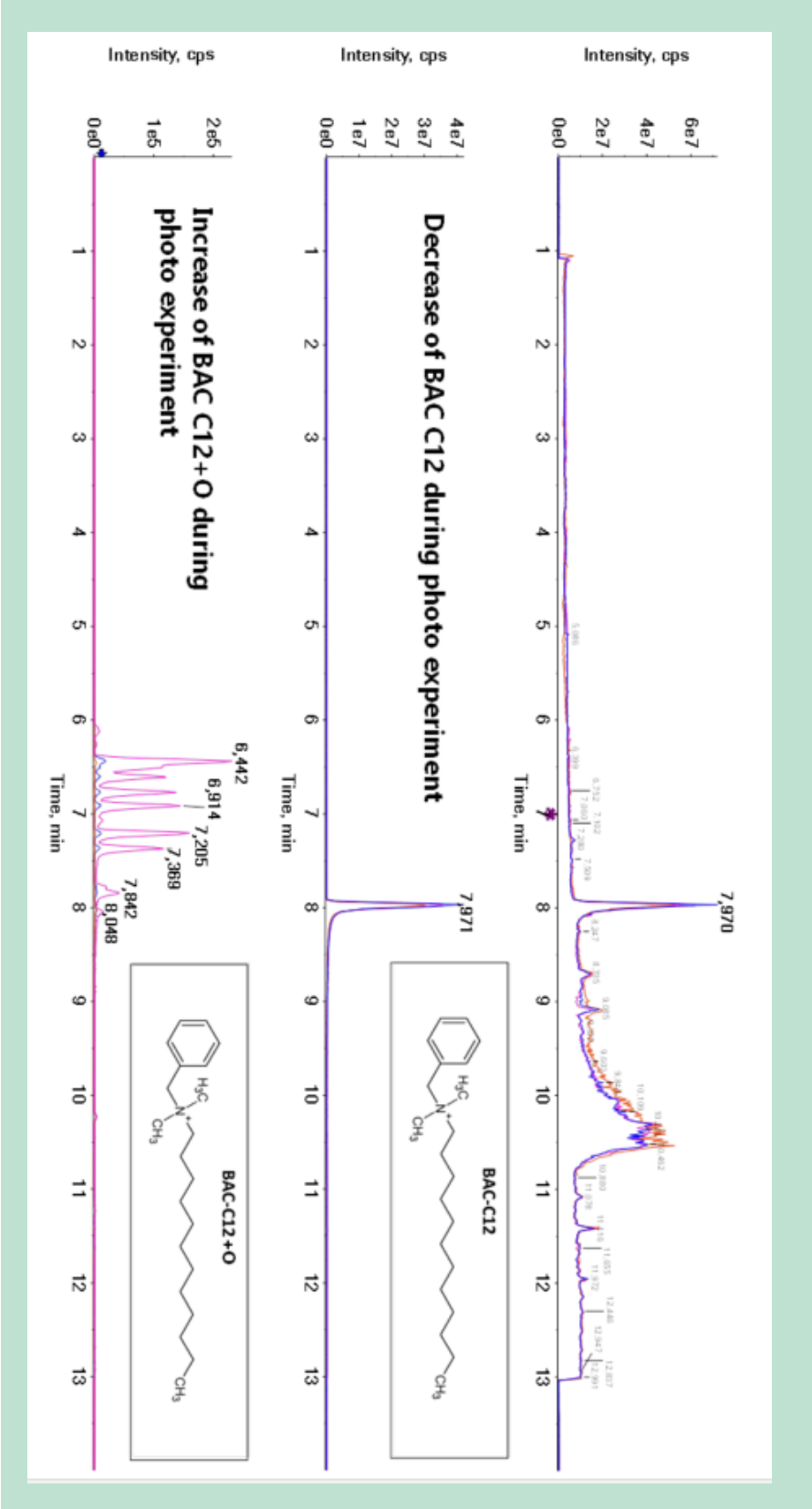


FIGURE 5.2. Photodegradation data of BAC-12.
All windows show the comparison photodegradation (blue), photodegradation with humic compounds (magenta) and dark incubation (red).
Up: Total Ion current (TIC) Sum of all signals detected.
Middle: Chromatogram of the parent BAC-12 (304.300 Da).

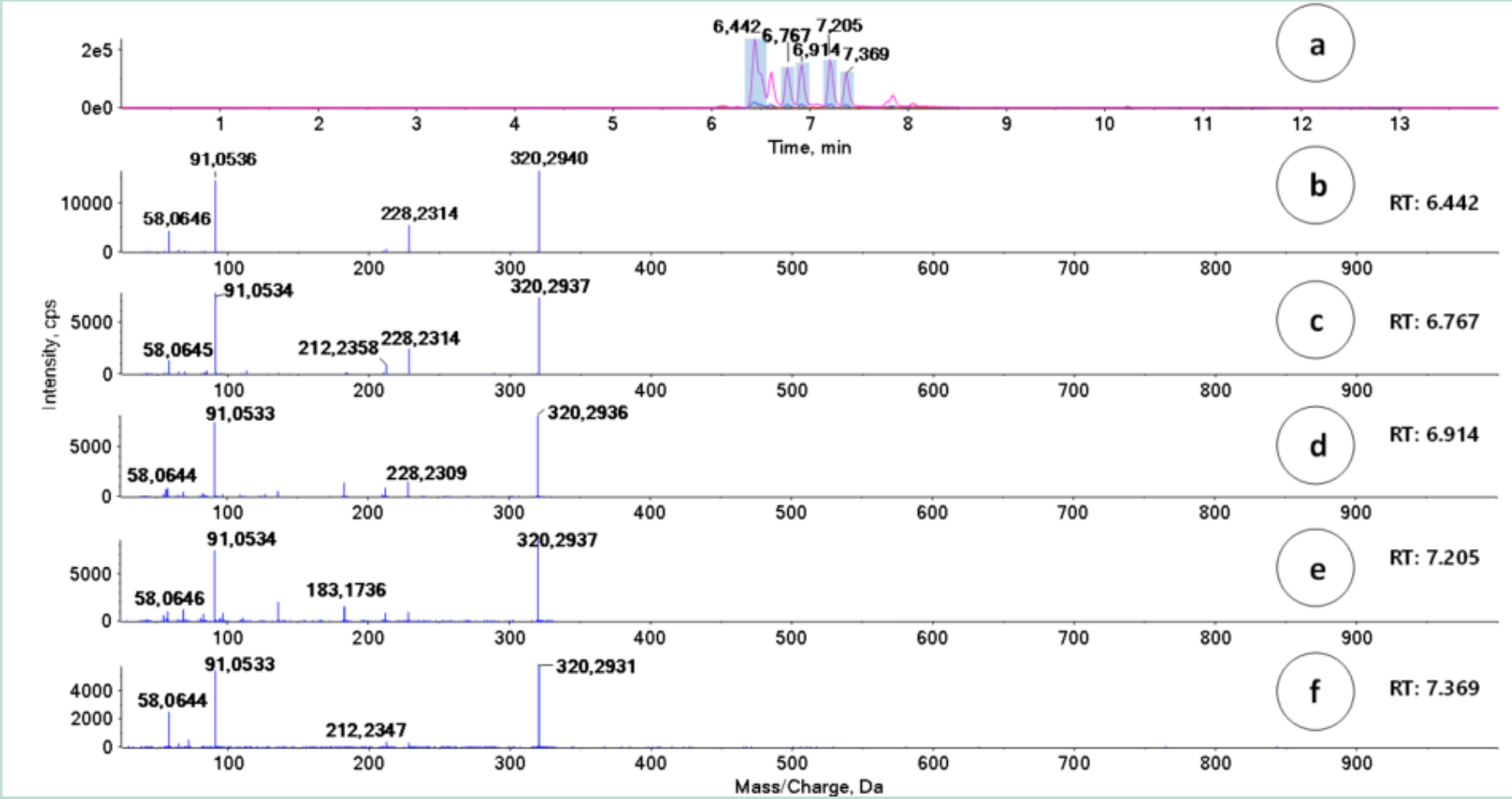

6,442
6,767
7,205
6,914
7,369
a
2e5
0e0
Time, min
b
91,0536
320,2940
58,0646
228,2314
RT: 6.442
c
91,0534
320,2937
58,0645
212,2358
228,2314
RT: 6.767
d
91,0533
320,2936
58,0644
228,2309
RT: 6.914
e
91,0534
320,2937
58,0646
183,1736
RT: 7.205
f
91,0533
320,2931
58,0644
212,2347
RT: 7.369
Intensity, cps
Mass/Charge, Da

FIGURE 5.3. a) XIC chromatogram of PTP320, b-f) Comparison of product ion spectra of 6 six isomers of the primary photooxidation products of BAC-12 (BAC-12+O)

5.1.2 BAC-12+O-H2 (BAC-12 PTP318)

Another photooxidation product was observed resulting from an addition of oxygen and abstraction of two hydrogen atoms. Mechanistically, this could be reached by forming aldehydes or ketones. Figure 5.4 exhibits the presence of four distinct chromatographic peaks. Several of the spectra were very much alike indicating several isomers. However, the number of isomers was less than for BAC-12 PTP320.This reaction can only take place in selected parts of the molecule (suggestions for structures are given in figure 5.4).

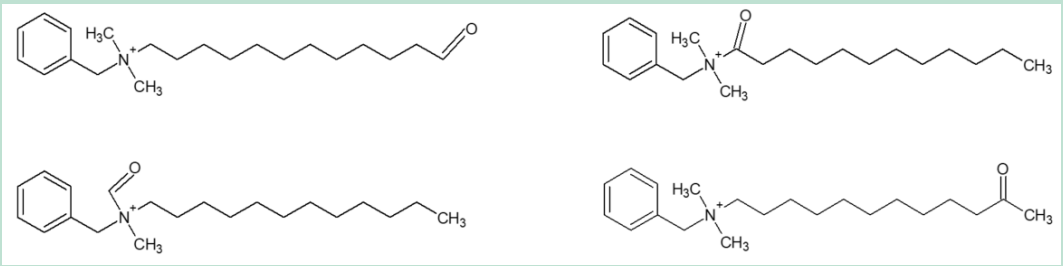


FIGURE 5.4. Suggestions for structures for BAC-12 PTP318.

While a lot of structural information can be obtained from the HPLC-HR MS/MS data, the single structure can only be annotated after isolation of the respective transformation product and obtaining NMR (nuclear magnetic resonance) spectra. However, it was not the intention or goal of this project to elucidate structures of phototransformation products, but to enable suspect screening for selected phototransformation products in environmental samples. – This is now possible and has successfully been conducted in selected samples (see Chapter 7).

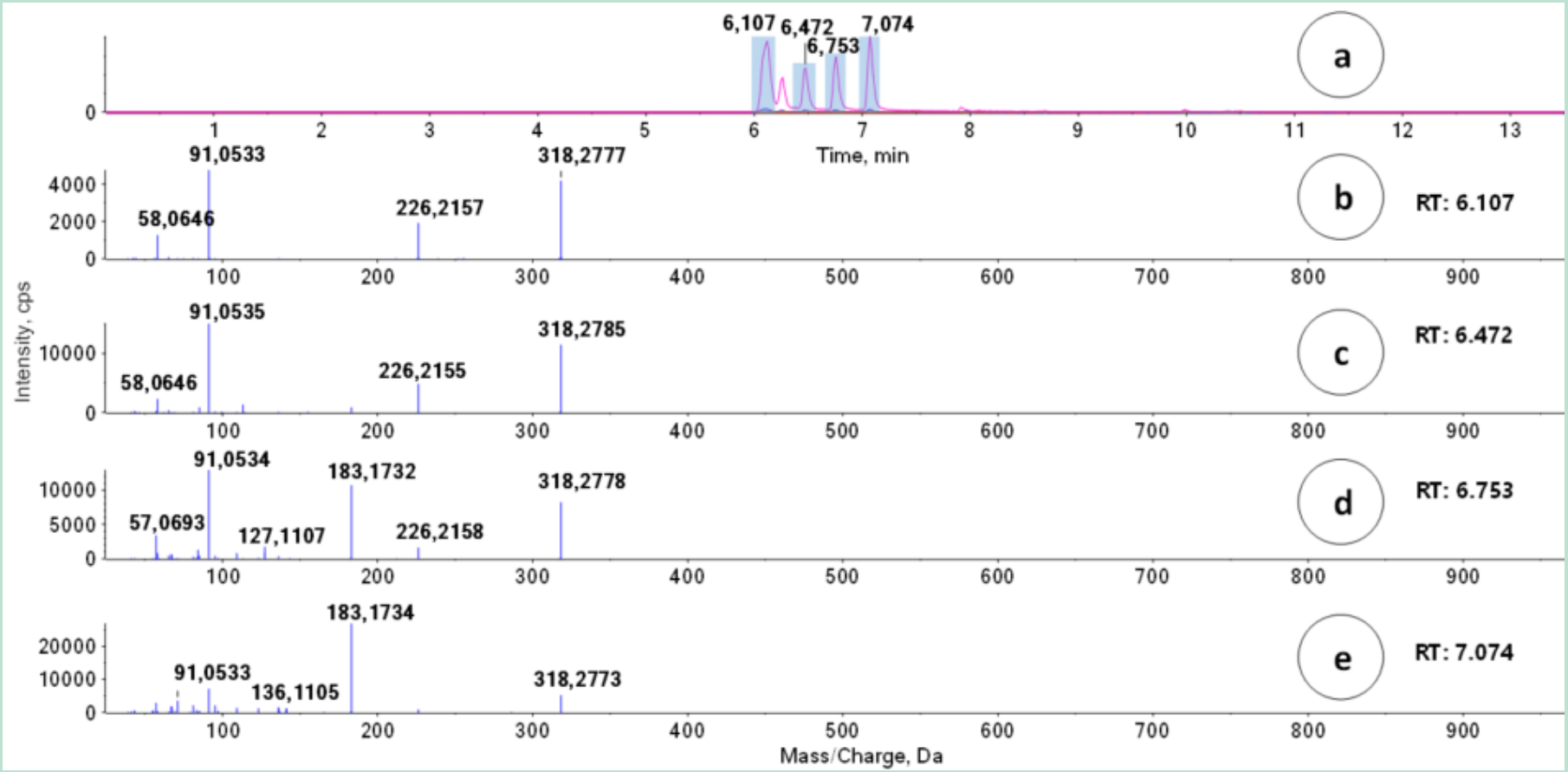

6,107
6,472
6,753
7,074
a
Time, min
91,0533
318,2777
58,0646
226,2157
b
RT: 6.107
Intensity, cps
91,0535
318,2785
58,0646
226,2155
c
RT: 6.472
91,0534
183,1732
318,2778
57,0693
127,1107
226,2158
d
RT: 6.753
183,1734
91,0533
136,1105
318,2773
e
RT: 7.074
Mass/Charge, Da

FIGURE 5.5. a) XIC chromatogram of PTP318, b-e) Chromatogram and product ion spectra of isomers of BAC-12 phototransformation product BAC-12 PTP318.

5.2 Biodegradation products

The nomination of the biotransformation products is similar to the phototransformation products, except the biotransformation products are nominated TP while the phototransformation products are nominated PTP. Interestingly, usually only one predominant biodegradation product was observed while other isomers were detected either not at all or with signals several orders of magnitude lower.

The biodegradation experiments were conducted with BAC-12 and BAC-14. It turned out that the degradation pathways were very similar and thus supporting each other. All degradation occurred as bio-oxidation (Figure 5.6 and Table 5.1).
Most degradation started with a ω (omega) oxidation, meaning the last carbon atom in the chain was oxidised leading to BAC-12 TP320.

BAC 12 TP320

This ω oxidation can be followed by a β oxidation (making acetate units available for the organism) and leading to chain shortening by 2 carbon atoms each. This leads to a high number of intermediates and metabolites (more than 20 have been described in the run of this project).

However, by this pathway only even chained transformation products can be synthesised, while also uneven chained oxidation products were observed. These can be biosynthesised by a second pathway that after the ω oxidation starts with an α oxidation, which results in the loss of one carbon atom, only. This α oxidation is then followed by a β oxidation, leading to the formation of a multitude of transformation products with alkyl chains with uneven number of carbon atoms.

Selected metabolites of BACs have already been described in the literature (Dean-Raymond & Alexander, 1977; Fortunato et al., 2019; Khan et al., 2015; Tezel et al., 2015 but this is to our knowledge the first time the pathway seems fully described with all metabolites.

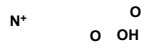
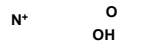
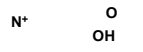
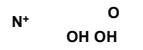
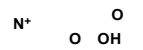
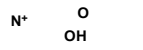
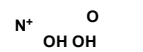
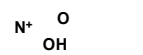
TABLE 5.1. Mass spectrometric characterisation of BAC-12 metabolites of the ω/β oxidation (Molecular ions (MS) and product ion spectrum (MS/MS)) of BAC-12 and its transformation products in the order of the degradation pattern. Retention times are given either for the reversed phase chromatography (RP) or hydrophilic interaction chromatography (HILIC). (Larsson et al., 2023)

Compound	Retention Time [min]	Confidence Level	MS or MS^2	Measured mass fragments [Da]	Suggested sum Formula	Mass deviation between detected and theoretical [mDa]*	Suggested Structure

>BAC-12	8.00	1	MS	**304.2962**	$C_{21}H_{38}N$	4.224		
	RP		MS^2	212.233	$C_{14}H_{30}N$	4.824		
				91.0513	C_7H_7	3.475		
				58.0626	C_3H_8N	3.074		
BAC-14	8.60	1	MS	**332.3293**	$C_{23}H_{42}N$	2.424		
	RP		MS^2	240.2681	$C_{16}H_{34}N$	1.024		
				91.0528	C_7H_7	1.975		
				58.0636	C_3H_8N	2.074		
TP320C	6.44	2	MS	**320.2975**	$C_{21}H_{38}NO$	2.161		
	RP		MS^2	228.2330	$C_{14}H_{30}NO$	0.261		
				91.0530	C_7H_7	1.775		
				58.0635	C_3H_8N	2.174		
TP318	6.06	2	MS	**318.2777**	$C_{21}H_{36}NO$	1.989		
	RP		MS^2	226.2157	$C_{14}H_{28}NO$	1.389		
				91.0528	C_7H_7	1.975		
				58.064	C_3H_8N	1.674		
TP334	5.38	2	MS	**334.2736**	$C_{21}H_{36}NO_2$	1.004		
	RP		MS^2	242.2114	$C_{14}H_{28}NO_2$	0.604		
				91.0532	C_7H_7	1.575		
				58.0631	C_3H_8N	2.574		
TP332	4.63	2	MS	**332.2558**	$C_{21}H_{34}NO_2$	3.154		
	RP		MS^2	240.1948	$C_{14}H_{26}NO_2$	1.554		
				91.0526	C_7H_7	2.175		
				58.0636	C_3H_8N	2.074		
TP348	4.97	2	MS	**348.2549**	$C_{21}H_{34}NO_3$	1.031		
	RP		MS^2	256.1890	$C_{14}H_{26}NO_3$	2.269		
				91.0524	C_7H_7	2.375		
				58.0630	C_3H_8N	2.674		
TP306B	5.54	2	MS	**306.2416**	$C_{19}H_{32}NO_2$	1.704		
	RP		MS^2	214.1789	$C_{12}H_{24}NO_2$	1.804		
				91.0529	C_7H_7	1.875		
				58.0641	C_3H_8N	1.574		
TP304	5.32	2	MS	**304.23**	$C_{19}H_{30}NO_2$	2.346		
	RP			212.1690	$C_{12}H_{22}NO_2$	3.946		
				91.0515	C_7H_7	3.275		
				58.0639	C_3H_8N	1.774		
TP322	4.24	2	MS	**322.2349**	$C_{19}H_{32}NO_3$	3.319		
	RP		MS^2	230.1737	$C_{12}H_{24}NO_3$	1.919		
				91.0522	C_7H_7	2.575		
				58.063	C_3H_8N	2.674		
TP320A	4.11	2	MS	**320.2212**	$C_{19}H_{30}NO_3$	1.369		
	RP		MS^2	228.1579	$C_{12}H_{22}NO_3$	2.069		
				91.0524	C_7H_7	2.375		
				58.0642	C_3H_8N	1.474		
TP278	4.40	2	MS	**278.2141**	$C_{17}H_{28}NO_2$	2.096		

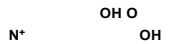
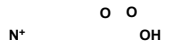
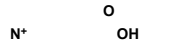
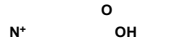
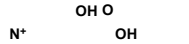
	RP	MS^2	186.1491	$C_{10}H_{20}NO_2$	0.304		
			91.0531	C_7H_7	1.675		
			58.0635	C_3H_8N	2.174		

TABLE 5.1 (continued) Mass spectrometric characterisation of BAC-12 metabolites of the ω/β oxidation (Larsson et al., 2023)

TP276	4.2	2	MS	**276.1978**	$C_{17}H_{26}NO_2$	1.446	
	RP		MS^2	184.1328	$C_{10}H_{18}NO_2$	0.954	
				91.0523	C_7H_7	2.475	
				58.0639	C_3H_8N	1.774	
TP294	3.54	2	MS	**294.2086**	$C_{17}H_{28}NO_3$	1.681	
	RP		MS^2	202.1415	$C_{10}H_{20}NO_3$	0.140	
				91.0525	C_7H_7	2.275	
				58.0640	C_3H_8N	1.675	
TP292A	3.40	2	MS	**292.1918**	$C_{17}H_{26}NO_3$	0.531	
	RP		MS^2	200.1278	$C_{10}H_{18}NO_3$	0.869	
				91.0522	C_7H_7	2.575	
				58.0621	C_3H_8N	3.574	
TP250	3.29	2	MS	**250.1795**	$C_{15}H_{24}NO_2$	1.204	
	RP		MS^2	158.1161	$C_8H_{16}NO_2$	2.004	
				91.0532	C_7H_7	1.575	
				58.063	C_3H_8N	2.674	
TP248	3.02	2	MS	**248.1652**	$C_{15}H_{22}NO_2$	0.146	
	RP		MS^2	156.1005	$C_8H_{14}NO_2$	1.954	
				91.0526	C_7H_7	2.175	
				58.0639	C_3H_8N	1.774	
TP266	1.52	2	MS	**266.1773**	$C_{15}H_{24}NO_3$	1.681	
	RP		MS^2	174.1132	$C_8H_{16}NO_3$	0.181	
				91.0532	C_7H_7	1.575	
				58.0633	C_3H_8N	2.374	
TP264B	1.51	2	MS	**264.1604**	$C_{15}H_{22}NO_3$	0.431	
	RP		MS^2	172.0939	$C_8H_{14}NO_3$	3.469	
				91.0520	C_7H_7	2.775	
				58.0612	C_3H_8N	4.474	
TP222A	1.09	2	MS	**222.1505**	$C_{13}H_{20}NO_2$	1.096	
	RP		MS^2	130.0856	$C_6H_{12}NO_2$	1.204	
				91.0555	C_7H_7	0.725	
				58.0634	C_3H_8N	2.274	
TP238	5.64	2	MS	**238.14**	$C_{13}H_{20}NO_3$	4.319	
	HILIC		MS^2	146.0808	$C_6H_{12}NO_3$	0.919	
				91.0535	C_7H_7	1.275	
				65.0388	C_5H_5	0.325	
TP194	4.94	2	MS	**194.1186**	$C_{11}H_{16}NO_2$	0.496	
	HILIC		MS^2	102.0541	$C_4H_8NO_2$	1.404	
				91.0537	C_7H_7	1.075	

				58.0648	C_3H_8N	0.874	

TABLE 5.1b. Mass spectrometric characterisation of BAC-12 metabolites of the α/β oxidation (Molecular ions (MS) and product ion spectrum (MS/MS)) of BAC-12 and its transformation products in the order of the degradation pattern. Retention times are given either for the reversed phase chromatography (RP) or hydrophilic interaction chromatography (HILIC). (Larsson et al., 2023)

Compound	Retention Time [min]	Confidence Level	MS or MS^2	Measured mass fragments [Da]	Suggested sum Formula	Mass deviation between detected and theoretical [mDa]*	Suggested Structure
TP348x	6.24	2	MS	**348.2576**	$C_{21}H_{34}NO_3$	3.731	
	RP		MS^2	256.1926	$C_{14}H_{26}NO_3$	1.331	
				212.1971	$C_{13}H_{26}NO$	4.339	
				91.0520	C_7H_7	2.775	
TP320B	4.92	2	MS	**320.2515**	$C_{20}H_{34}NO_2$	7.454	
	RP		MS^2	228.1959	$C_{13}H_{26}NO_2$	0.454	
				91.0524	C_7H_7	2.375	
				58.0652	C_3H_8N	0.474	
TP292B	4.97	2	MS	**292.229**	$C_{18}H_{30}NO_2$	1.346	
	RP			200.1633	$C_{11}H_{22}NO_2$	1.754	
				91.0525	C_7H_7	2.275	
				58.0633	C_3H_8N	2.374	
TP308	3.76	2	MS	**308.2197**	$C_{18}H_{30}NO_3$	2.869	
	RP			216.1589	$C_{11}H_{22}NO_3$	1.069	
				200.1655	$C_{11}H_{22}NO_2$	0.446	
				91.0524	C_7H_7	2.375	
TP306A	3.76	2	MS	**306.2042**	$C_{18}H_{28}NO_3$	2.719	
	RP		MS^2	214.1423	$C_{11}H_{20}NO_3$	2.019	
				171.0995	$C_9H_{15}O_3$	2.620	
				125.0941	$C_8H_{13}O$	2.540	
				91.0529	C_7H_7	1.875	
TP264A	3.82	2	MS	**264.195**	$C_{16}H_{26}NO_2$	1.354	
	RP		MS^2	172.1326	$C_9H_{18}NO_2$	1.154	
				91.0536	C_7H_7	1.175	
				65.0375	C_5H_5	1.625	
				58.0645	C_3H_8N	1.174	
TP262	3.62	2	MS	**262.178**	$C_{16}H_{24}NO_2$	2.704	
	RP			170.1177	$C_9H_{16}NO_2$	0.404	
				91.053	C_7H_7	1.775	
				58.0641	C_3H_8N	1.574	
TP280	2.71	2	MS	**280.191**	$C_{16}H_{26}NO_3$	0.269	
	RP		MS^2	188.1280	$C_9H_{18}NO_3$	0.669	
				91.0525	C_7H_7	2.275	

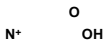
				58.0629	C_3H_8N	2.774	
TP236B	5.935	2	MS	**236.164**	$C_{14}H_{22}NO_2$	1.331	
	HILIC		MS^2	144.1011	$C_7H_{14}NO_2$	1.354	
				91.0531	C_7H_7	1.675	
				58.0643	C_3H_8N	1.374	
TP208B	5.96	2	MS	**208.133**	$C_{12}H_{18}NO_2$	0.754	
	HILIC		MS^2	116.0709	$C_5H_{11}NO_2$	0.254	
				91.0537	C_7H_7	1.075	
				58.0649	C_3H_8N	0.774	

TABLE 5.1c. Mass spectrometric characterisation of BAC-12 metabolites from unidentified pathways (Mo-lecular ions (MS) and product ion spectrum (MS/MS)) of BAC-12 and its trans-formation prod-ucts in the order of size. Retention times are given either for the reversed phase chromatog-raphy (RP) or hydrophilic interaction chromatography (HILIC).

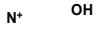
Com-pound	Reten-tion Time [min]	Confi-dence Level	MS or MS^2	Measured mass frag-ments [Da]	Suggested sum For-mula	Mass de-viation between detected and theo-retical [mDa]*	Suggested Structure
TP336	5.42	3	MS	**336.2914**	$C_{21}H_{38}NO_2$	1.146	
	RP		MS^2	244.2279	$C_{14}H_{30}NO_2$	0.246	
				91.0532	C_7H_7	1.575	
				58.0639	C_3H_8N	1.775	
TP236A	3.35	3	MS	**236.2022**	$C_{15}H_{26}NO$	0.761	
	RP		MS^2	144.1379	$C_8H_{18}NO$	0.939	
				91.0522	C_7H_7	2.575	
				58.0640	C_3H_8N	1.674	
TP222B	5.33	3	MS	**222.1838**	$C_{14}H_{24}NO$	1.989	
	HILIC		MS^2	130.1231	$C_7H_{16}NO$	0.089	
				91.0541	C_7H_7	0.675	
				58.0650	C_3H_8N	0.674	
TP208A	3.35	3	MS	**208.1682**	$C_{13}H_{22}NO$	1.939	
	HILIC		MS^2	116.1072	$C_6H_{14}NO$	0.339	
				91.0540	C_7H_7	0.775	
				58.0647	C_3H_8N	0.974	
TP152	5.444	1	MS	**152.106**	$C_9H_{14}NO$	1.539	
	HILIC		MS^2	91.0559	C_7H_7	1.125	
				65.0380	C_5H_5	1.125	
				39.0226	C_3H_3	0.875	

FIGURE 5.6 Postulated degradation of BAC 12 by ω, β and α oxidation. All metabolites in black are detected by HPLC HRMS with product ion spectra at confidence level 2 or higher. (Larsson et al., 2023)

5.3 Key findings on QUATs degradation and degradation products

- Both photodegradation and biodegradation result in detectable transformation products.
- More than 35 metabolites (biotransformation products) were characterised from BAC-12 alone.
- More than 16 phototransformation products were characterised from BAC-12 alone.
- The phototransformation usually resulted in a multitude of isomers (as expected).
- The biodegradation usually resulted in only one or max three isomers with one being prevalent.
- Photodegradation was enhanced in the presence of humic compounds.

Based on these experiments it can be stated that degradation products of QUATs are expected in the environment and their relevance (toxicity) needs to be assessed.

6. Market survey (WP2)

Based on the criteria “cationic tensides” or the direct mentioning of quaternary ammonium compounds on the labels, 32 products were identified as potentially QUAT containing. In these there were 1) disinfectants for human hygiene (PT 1), 2) laundry softeners/detergents, and 3) indoor/outdoor cleaners.

To assess this, PT 1 and laundry products were screened in supermarkets Meny and Kvickly, and products for treating roofs and tiles (Tagrens & Fliserens) were searched for in the do-it-Yourself markets “XL” and “Johannes Fog” in spring 2021.

A selection of products is visible in figure 6-1.

FIGURE 6.1. Selection of compounds that were tested for biocidal QUATs.

All products were analysed by HPLC coupled to high resolution mass spectrometry in comparison to standards (Table 6-1)

TABLE 6.1. Overview on tested products with confirmed ingredients.

		DDAC	BAC						ATMAC						TDAC			
Description	QUATs (label)	10	6	8	10	12	14	16	8	10	12	14	16	18	8	10	12	Comment
Rodalon skimmel 1L	7% DDAC-10	X			X	X	X	X		X		X			X			

Weibulls perfekt tak										X			
Ecostyle algefri										X			
Rodalon fjerner belægninger udendørs	<0.1% DDAC-C10									X			
Rodalon dræber bakterier indendørs	BAC+DDAC <2.5%	X								X			
Nilfisk stone & wood cleaner										X			
Exo fliserens										X			
Borup afrenser	< 15% cationic surfactants				X	X				X			
Neutralon algæfjerner	0.5% DDAC	X								X			
Juliana surface cleaner		X			X			X		X		X	
Borup fliserens										X			
Gori ekstra										X			
Hornum fliserens	< 5% cationic surfactants									X			
Jotun Træ og murerens	BAC			X	X	X	X			X			
Hornum belægningsrens	5-15% cationic surfactants			X	X	X				X			
Sadolin Husrens	15-30% cationic surfactants			X	X	X				X			
Rodalon klar til brug desinfectant	<0.25% BAC	X		X	X	X	X	X		X			
Jotun 2 in 1 husvask/træ og murerens	1-10% cationic surfactants	X		X	X	X		X	X	X			
Gori fjern belægninger	5-15% cationic surfactants				X	X				X			
Exo belægnsrens	< 5% cationic surfactants									X			
Borup nåletræ terrasse rens										X			
Sadolin husrens 2.5L	<5% cationic surfactants			X	X	X				X			
Mellerud belægningsfjerner	BAC+QAC			X	X	X	X			X			
Junckers outdoor										X			
Levevis skyllemiddel										X			Laundry detergent

TABLE 6.1. (continued) Overview on tested products with confirmed ingredients.

		DDAC	BAC						ATMAC						TDAC			
Description	QUATs (label)	10	6	8	10	12	14	16	8	10	12	14	16	18	8	10	12	Comment
Dunlet ekstra	< 5% cationic surfactants											X						Laundry detergent
noora	5-15% cationic surfactants											X						Laundry detergent
Bamseline creations	5-15% cationic surfactants											X						Laundry detergent
salling skyllemiddel	5-15% cationic surfactants											X						Laundry detergent
Bamseline blid og blød	5-15% cationic surfactants											X						Laundry detergent
Neutral fabric conditioner	5-15% cationic surfactants											X						Laundry detergent
Ecover	5-15% cationic surfactants											X						Laundry detergent

A qualitative crosscheck on the roof cleaning/terrace cleaning products in do-it-Yourself-markets "Johannes Fog" and "XL" in 2023 gave the impression, that of the majority of products biocidal QUATs have now been replaced by pelargonic acid (*n*-nonanoic acid). Even though this project does not focus on risk assessments, this is probably an improvement for the environment.

6.1 Key findings on QUATs Market survey

- The classical PT 2 products (Rodalon) indeed contained QUATs – sometimes BACs on top of the declared DDAC.
- Especially BAC-10, -12 and -14 are predominant compounds in most of the examined products.
- "Cationic surfactants" could often be translated into biocidal QUATs, usually as mixtures of BAC-10, -12, -14.
- Though most of the roof cleaning and terrace cleaning products contained biocidal QUATs, but not all of them did.
- None of the laundry products contained QUATs that are under discussion in the biocidal product regulations (BPR (EU, 2012)).

7. Emissions of QUATs via urban surface water runoff (WP3)

7.1 Emission concentrations

For assessing the QUATs present in runoff water, 168 samples of runoff water were collected in a sub-catchment of Silkeborg over two years and were analysed successively. Usually BAC-12, -14 and -16 as well as DDAC-10 were detected, while other congeners were only detected in single samples at very low levels. The found concentrations are shown in Figure 7.1 (for 2021) and Figure 7.2 (for 2022). The concentrations ranged from a few ng/L for each compound up to 9633 ng/L for BAC-12, 3420 ng/L for BAC-14, 1245 ng/L for BA-16 and 2940 ng/L for DDAC. From the results, it is clear that the presence of QUATs into the runoff was not constant over time and it was not well correlated to rain events. This could be due to timing of the usage of QUATs over the seasons. Indeed, the higher values of QUATs were found in the sampling of July and the summer is the season where most people clean roofs and/or terraces of their houses. It is also interesting to note that on average, lower values were found in 2022 than in 2021. The explanation could be that in the last year many roof cleaning products have begun to replace QUATs with other active compounds, such as nonanoic acid (see Chapter 6).

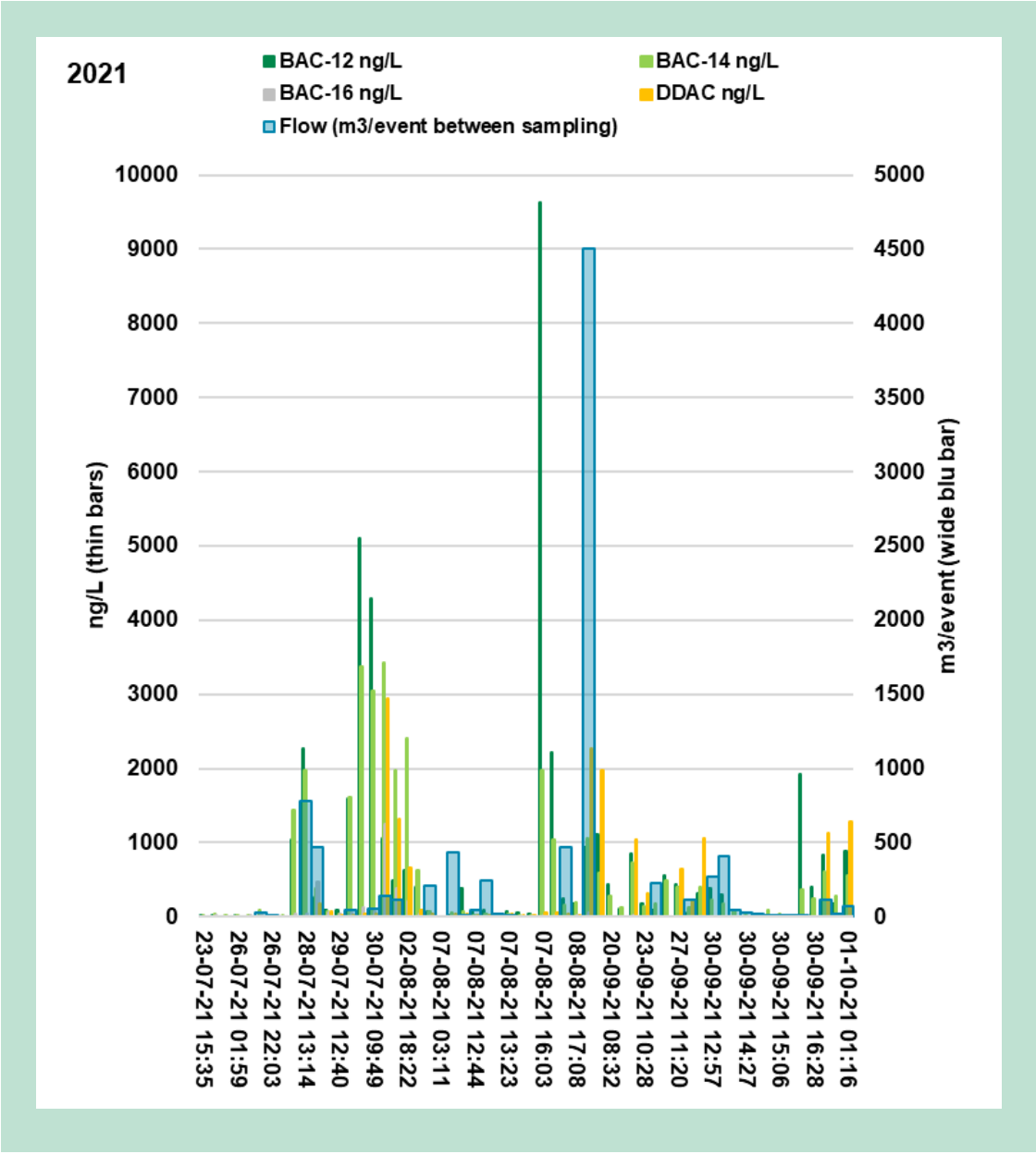


FIGURE 7.1. Concentrations of the predominant QUATs in relation to the volume of water per runoff event in samples from 2021 in the Silkeborg stormwater catchment.

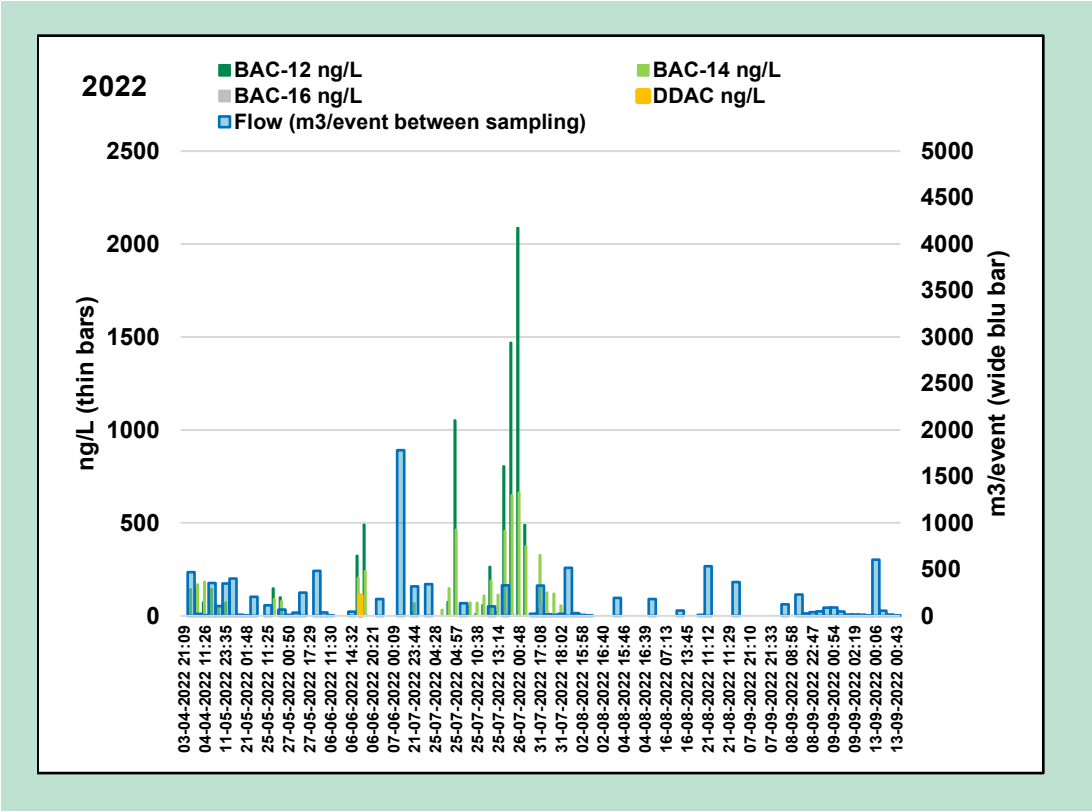


FIGURE 7.2. Concentrations of the predominant QUATs in relation to the volume of water per runoff event in samples from 2022 in the Silkeborg stormwater catchment.

Considering the environment it needs to be stated that there was one event in which concentrations above the EC_{50} for fish was determined in samples from the stormwater sewer. Thus even these relatively low emissions with no indications towards a real fresh treatment of a larger area (worst case scenario) can lead to problematic concentrations at least at the discharge point of the pipes.

7.2 Emission mass loads

To obtain a clearer picture and to be able to link to possible sources, the concentrations (C) were transferred into mass loads (M) by multiplying the concentrations by the runoff volume.
The emission mass loads of QUATs are show in Figure 7.3 and Figure 7.4. The emissions mass loads are in the range of grams for each event.
Even though most events are dominated by the same pattern of BAC-12, -14 and -16. Also, events with quite different patterns were recorded. This is indicating a significant diversity of sources.

In 2021 two major emissions were detected. On the 28th of August 1.8 g of BAC-12, 1.5 g of BAC-14 were detected. On 16th of September 4.2 g of BAC-12, 4.7 g of BAC-14 and 10.2 g of DDAC were detected in a single event.
In 2022 all the emission mass loads were lower in comparison to 2021. The major emissions occurred on the 26th of July with 0.3 g of BAC-12 and 0.2 g of BAC-14.
These data were compared to two scenaria: A) current usage during the experiment and B) derelict usage and emissions from areas that have been treated months/years ago:

A) Current usage considerations:

However, considering that a roof is treated with 10-30 L of a 0.5% solution (Table 6-1), (i.e., 50 to 150 g active ingredient, assuming authorised usage), the comparison with the observed mass flows in the monitored time periods are about a factor 10 too low to be caused by fresh roof applications dumped 100% into the stormwater sewers. The findings in the stormwater sewers might indicate towards ongoing applications with a lower transfer rate (10%) into the stormwater sewers while 90% remains sorbed to the roofs or is (partially) infiltrated into the soil before discharge to the stormwater sewers. This is supported by the fact that whole roof treatments would be expected to lead to one high emission followed by a slow decline of relatively lower concentrations which is neither visible in the concentrations, nor in the mass balances.

The observed pattern might rely to smaller applications as typically occurring for terraces, or applications that mostly infiltrate into soil and only a small fraction entering the storm sewers (again terraces). However, the one-time emission of 10 g DDAC-10 and close to 12 g BAC 12 might be due to a single application close to the stormwater sewers or a disposal or residual material into the stormwater sewers.

The observation that both events with high BAC and DDAC where determined indicated that both emissions from both surface cleaning agents (BAC) and wood protection (DDAC-10) were relevant in the catchment in different events.

B) Derelict usage (months/years before the experiment)

Assuming most of the BAC emissions originated from areas treated several years previously, would lead to an expectation of more constant loads and concentrations in each event (as it was found when analysing runoff from treated material (Gromaire et al., 2015).

In previous studies on biocides in renders and paints, it could be resolved with other environmental factors such as driving rain were determining the emissions of biocides (Bollmann et al., 2014). In which way these control the emissions of QUATs was not topic of this project and remains unresolved at this moment.

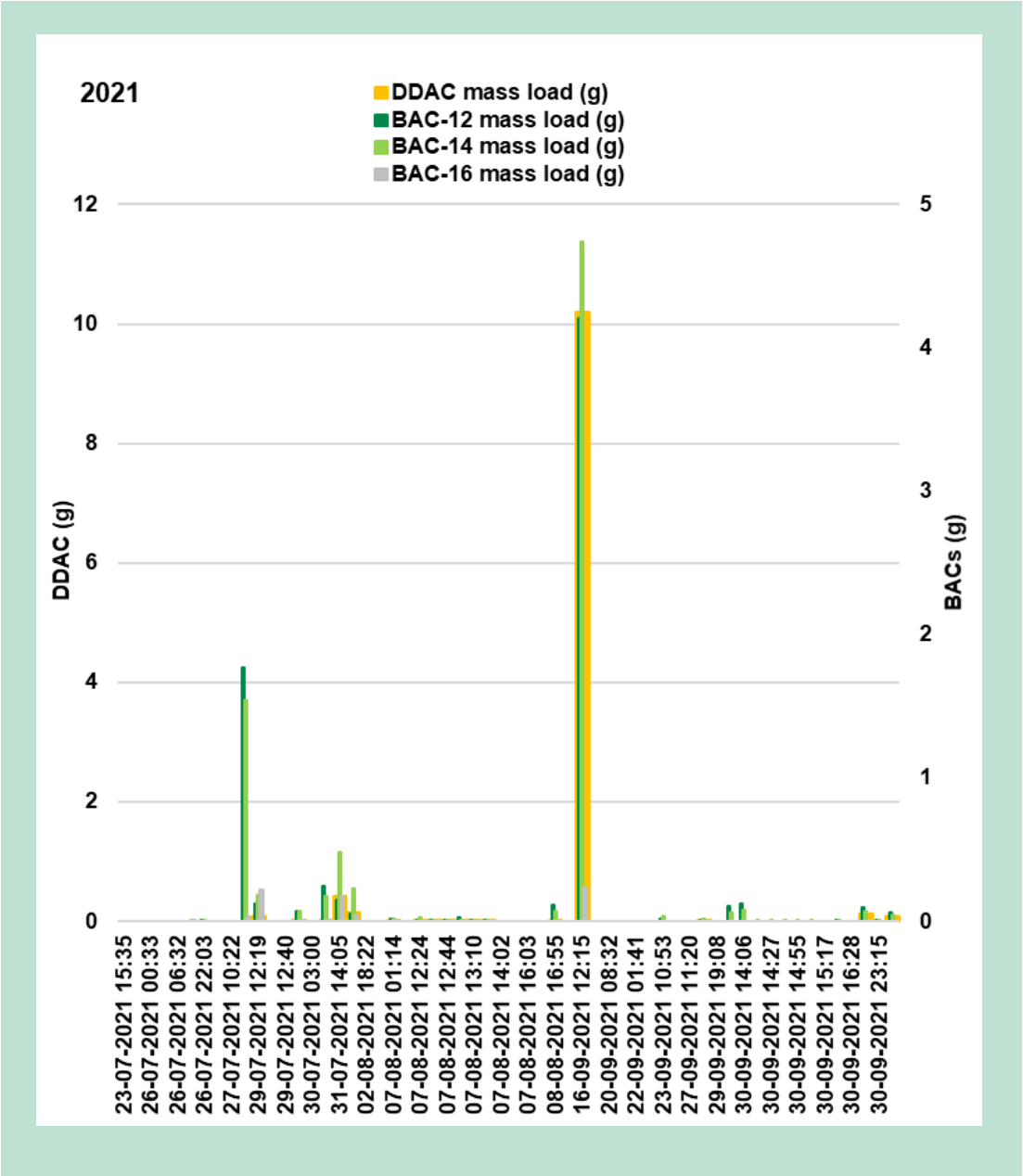


FIGURE 7.3. QUATs emission mass loads in 2021 in the Silkeborg stormwater catchment.

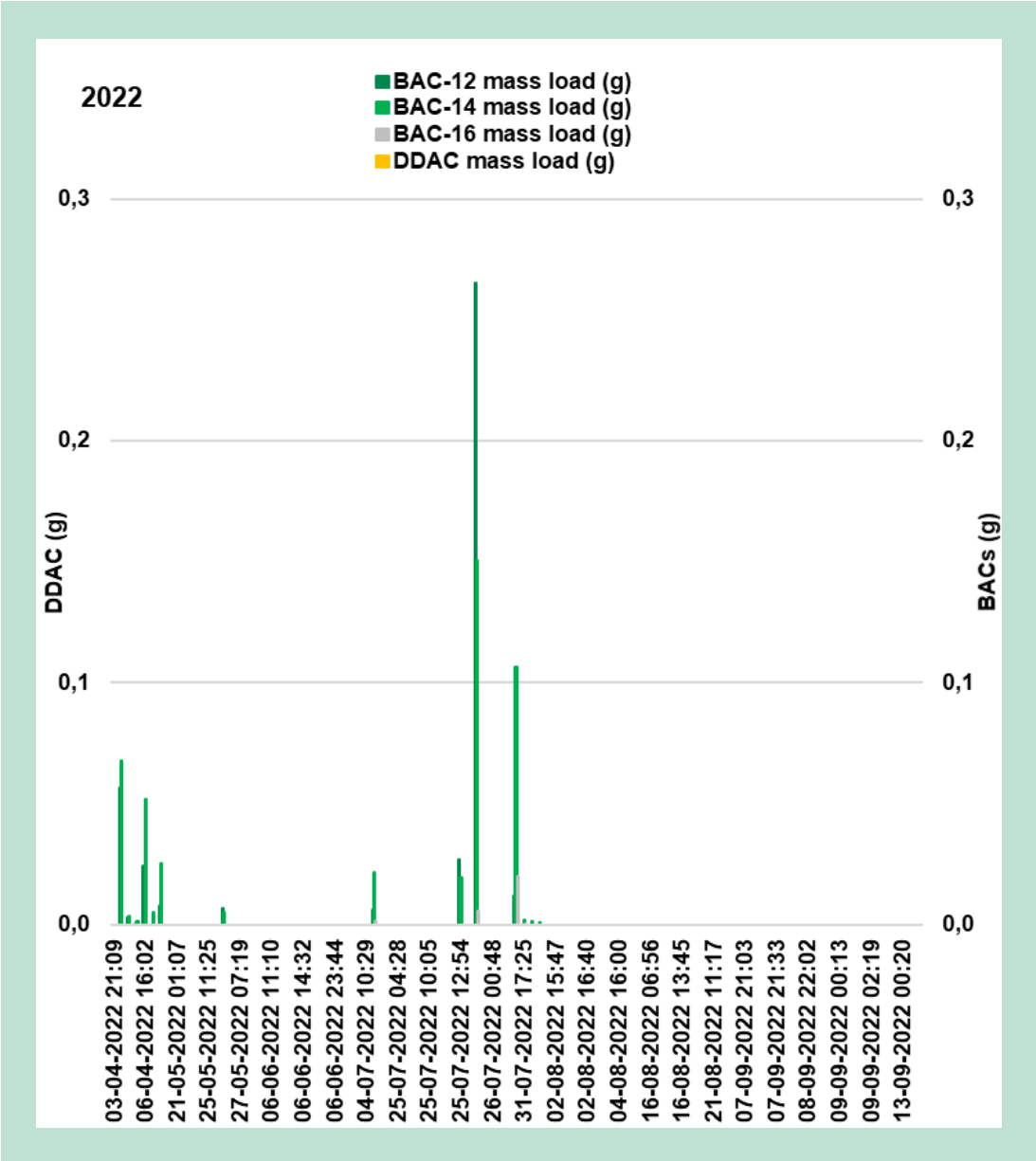


FIGURE 7.4. QUATs emission mass loads in 2022 in the Silkeborg stormwater catchment.

7.3 Transformation products in stormwater

Selected surface runoff samples were analysed the same way as described for the metabolite identification. Mass spectra and retention times were compared to the products found in the biodegradation and photooxidation experiments as those were not available as commercial standards. It turned out that the metabolites BAC-12 TP250, TP248, and BAC-12 TP *N,N* dimethyldecylamine N-oxide were determined in several samples (see overlay in Figure 7.5 to 7.7) additionally also photodegradation products of DDAC 10 were determined (Figure 7.8). These results make it more probable, that the finding of QUATs in surface runoff water in reality results from complex environmental processes such as soil passage in which biodegradation is possible, rather than from emissions from direct sources. – The presence of photodegradation does not imply much, as photodegradation easily can occur both on roofs and terraces after application.

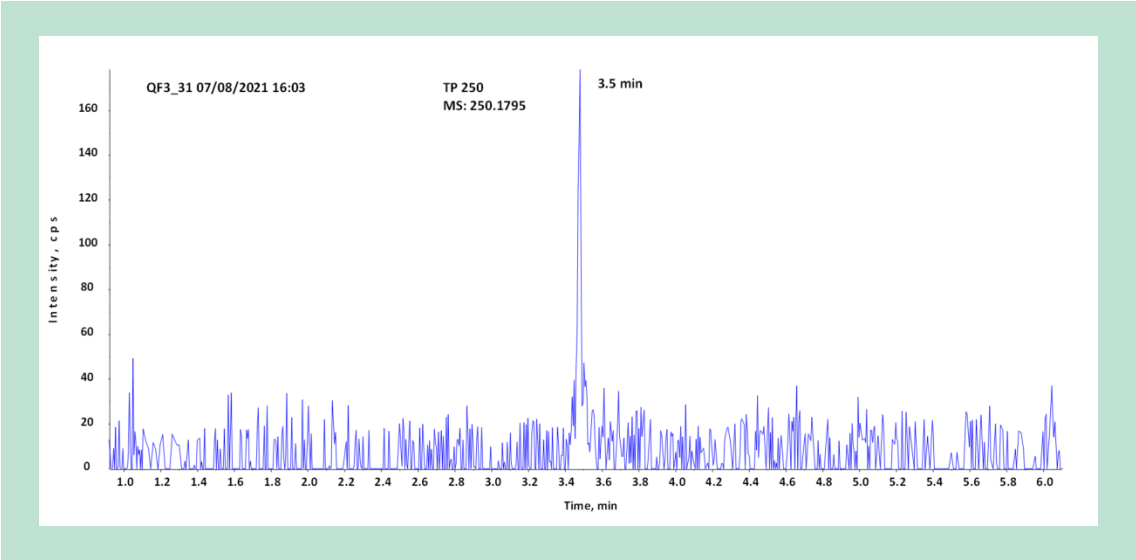


FIGURE 7.5. Chromatogram of BAC-12 TP250 detected in stormwater samples, RT and spectra confirmed with metabolites originating from a biodegradation experiment (Table 5-1).

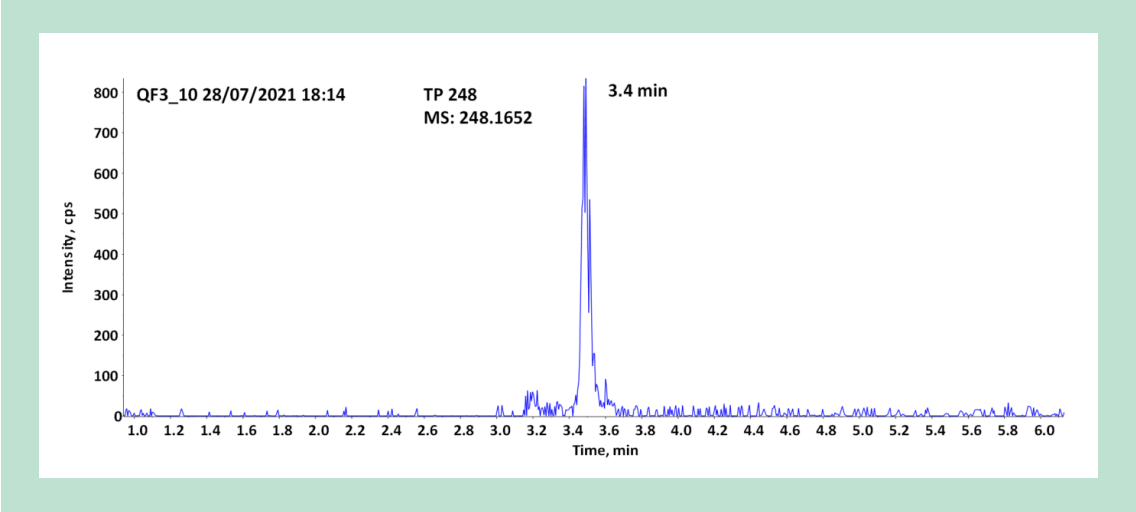


FIGURE 7.6. Chromatogram of BAC-12 TP248 detected in stormwater samples, RT and spectra confirmed with a true standard.

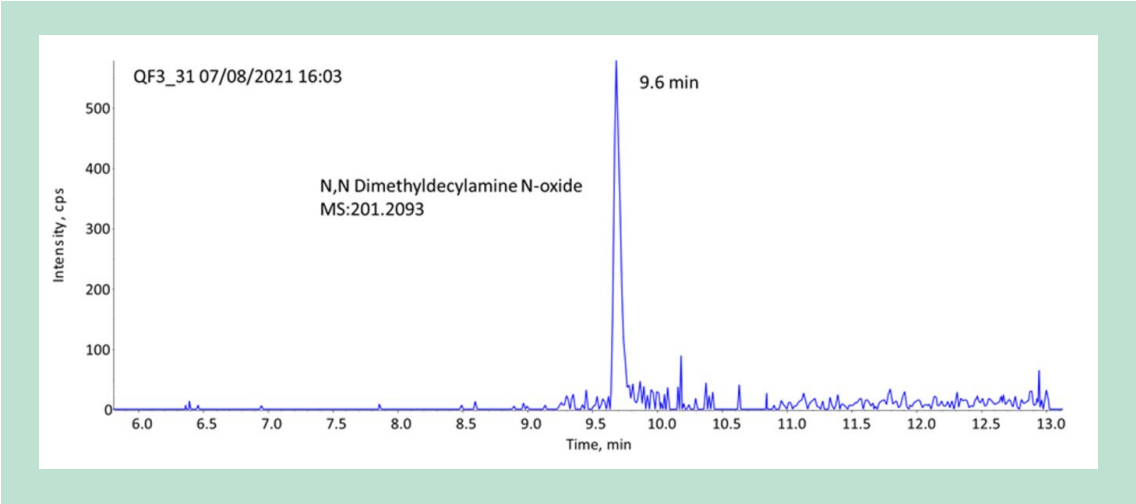


FIGURE 7.7. Chromatogram of BAC-12 TP N,N-dimethyldecylamine N-oxide (different isomers) detected in stormwater samples, RT and spectra confirmed with a true standard.

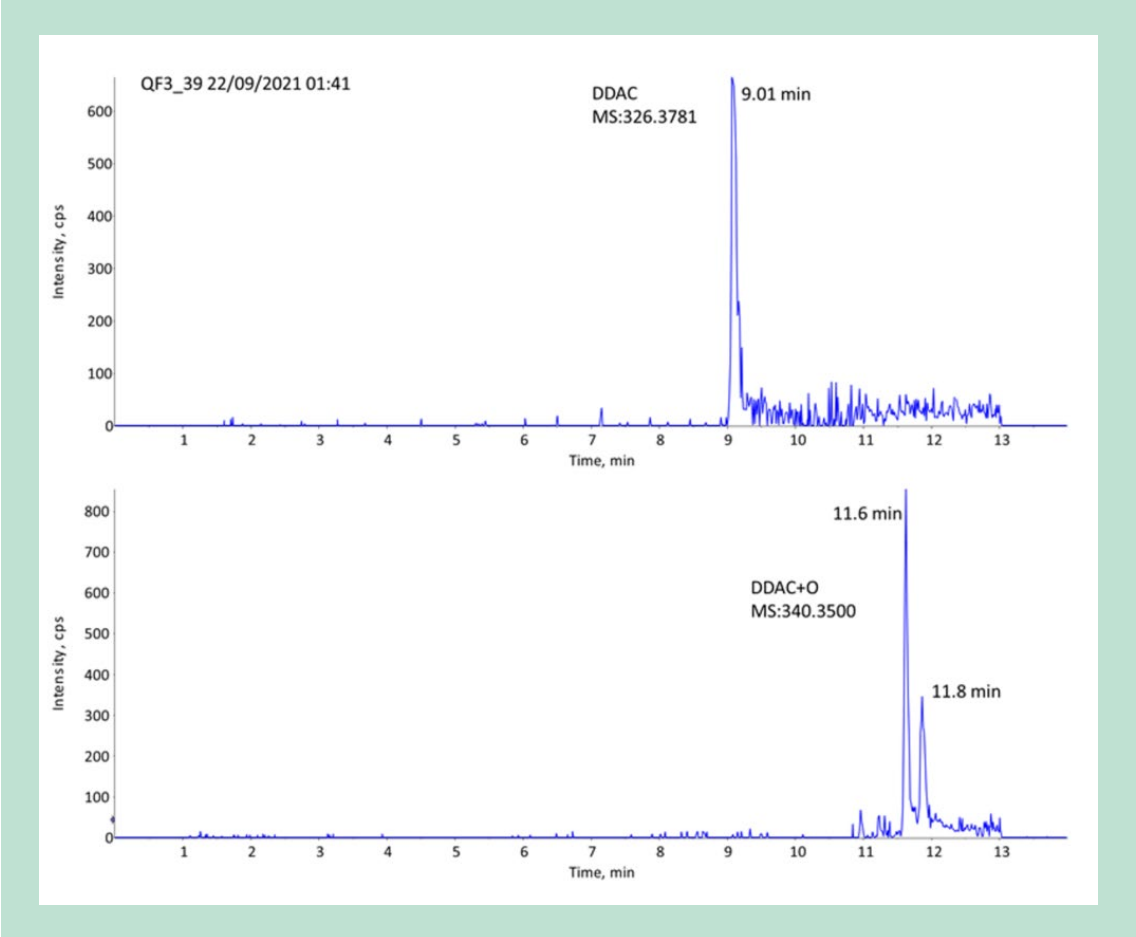


FIGURE 7.8. Chromatogram of DDAC-10 (top) and DDAC-10+O photodegradation product isomers (below) confirmed with samples from the photodegradation experiment (Analogue to Figure 5.3).

7.4 Discussions on QUATs in stormwater

The occurrence of QUATs in surface runoff waters is corroborated by the findings of Gromaire et al. (2015) regarding the concentrations observed in runoff from treated roofs. The determined

mass loads do not reflect expected emissions from a full-scale roof treatment that was directly (100%) discharged into the stormwater sewers, but rather to i) a series of smaller applications/discharges ii) a full scale applications with transfer rate of 10% to the sewer due to sorption of 90% of the applied material or iii) a full scale applications with transfer rate of 10% to the sewer due to most of the discharge water was infiltrated into the soil and not discharged to the stormwater sewers. Which of these discharges are relevant remains unresolved. Generally speaking, also the environmental factors controlling the emissions are not clear. Neither of this was the focus point of this project. However, the high abundance of signals from photodegradation products is striking.

7.5 Key findings on QUATs in surface water runoff.

- QUATs were detected in surface water runoff (stormwater sewers) in high but usually sub-toxic concentrations in the Silkeborg catchment.
- Obviously, none of the occasionally observed worst case applications, i.e., quantitative discharge of BACs after application on roofs during first rain event has been detected in the Silkeborg experiments.
- Both parent compounds, metabolites and photooxidation products are relevant in surface water runoff.
- The mechanisms controlling the emissions are not clarified, at the moment.

FIGURE 7.9. Professionals cleaning roofs of a building complex
(It is unknown which biocides were used during this individual operation).

8. Fate of QUATs in wastewater treatment plants (WP4)

8.1 Sorption to sludge

Description of test

Tests were conducted as partitioning to secondary sludge which was taken from Bjergmarken WWTP. For the sorption, the sludge was biologically inhibited by adding sodium azide (see 3.6.1.2 and 3.6.1.3).

All partitioning experiments were conducted on multiple concentrations in parallel to a blank (pure water) partitioning experiment.

Results:

While the short-chained compounds (BAC-6 and ATMAC-8) are mainly contained in the water, the longer chained compounds (with docdecyl groups or higher) are mostly sorbed to the sludge, as expected (See Figure 8-1 as example). The very high partitioning to the sludge for the larger molecules, however, was surprising. In Table 8.1 the numerical data for the sorption are presented.

TABLE 8.1. Partitioning constants (KD) for QUATs from the sludge water partitioning experiments in com-parison to the partitioning to organic material (KOC) and calculated octanol water partitioning constants (KOW).

Compound	K_D	$logK_D$	lgK_{OC}	lgK_{OW}
	[L/Kg]	[lg(L/Kg)]	[lg(L/Kg)]	[lg(L/Kg)]
BAC-6	4.3	0.61	0.77	0.39
BAC-12	1914	3.0	3.4	4.1
BAC-14	11153	3.8	4.2	5.1
BAC-16	32048	3.9	4.7	5.8
DDAC-10	8011267	6.3	7.0	9.1
ATMAC-8	2.1	0.32	2.9	-0.03
ATMAC-12	37	1.6	1.7	1.7
ATMAC-14	567	2.7	2.9	3.3
TDAC-8	400	2.5	2.7	3.1
TDAC-12	1730	3.0	3.4	4.0

In this report not only the final partitioning constants that rely on the relation concentration in the aqueous versus the concentration in the solids (C_a/C_s) is given, but also an analysis of the mass fractions in relation to the added (shown on the x axis) is given (Figure 8.1 to Figure 8.5), to ensure good understanding on the quality of data.

Data are usually presented as mass balances (%) in relation to the spiked amount.

High and above 100% fractiles for low concentrations (as seen for BAC-6) are an indication for high concentrations in the original sludge (as there is no sludge without any QUATs). – The C_a/C_s would not be affected, and the data can be used. However, blanks can influence especially the concentration dependency of the C_a/C_s ratio. Thus, the concentration dependency of the C_a/C_s is also analysed and for the final data only those values agreeing with the high concentrations are used (this analysis is not shown).

Stable but below 100% fractiles (as seen for BAC-12) are an indication for incomplete extractions from the sludge, this is important to memorise, but the effect on the logarithmic final values (log K_D) is small.

Low fractiles for the positive control (low recovery) (as seen for BAC-16) indicates towards sorption to the glass, which could usually be overcome by washing the glass with methanol acidified with formic acid. Usually, the mass balance for these compounds is dominated by the sorption to sludge and the log K_D is hardly affected.

Low fractiles for low concentrations and higher fractiles for higher concentrations usually indicate towards operations close to detection limits. In this case only the high concentrations are considered.

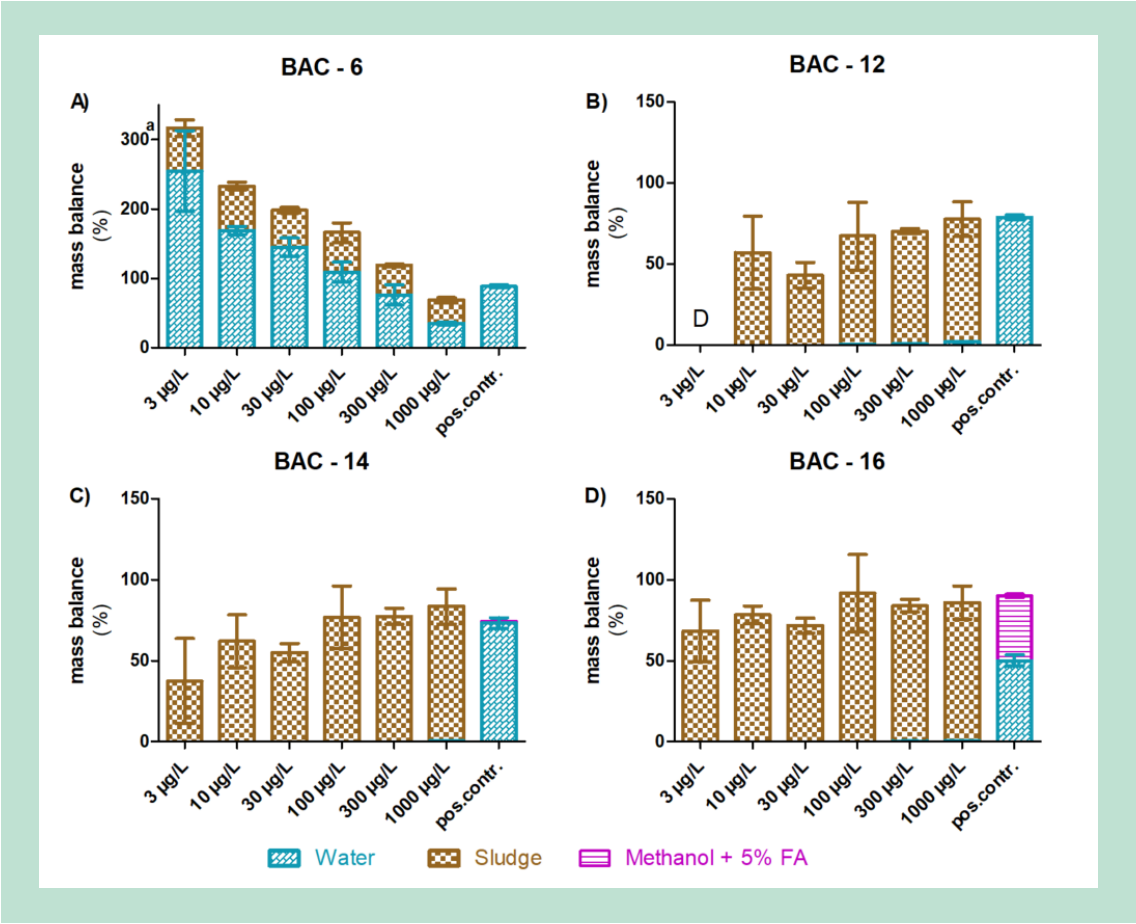


FIGURE 8.1. Exemplary raw data on partitioning of BACs to secondary natural sludge. The results are given in relation to the spiked amount (C/C0). If values higher than 100% are observed, the BAC contribution from the initial sludge was considerable. The positive control is QUATs spiked not in sludge, but into pure water.

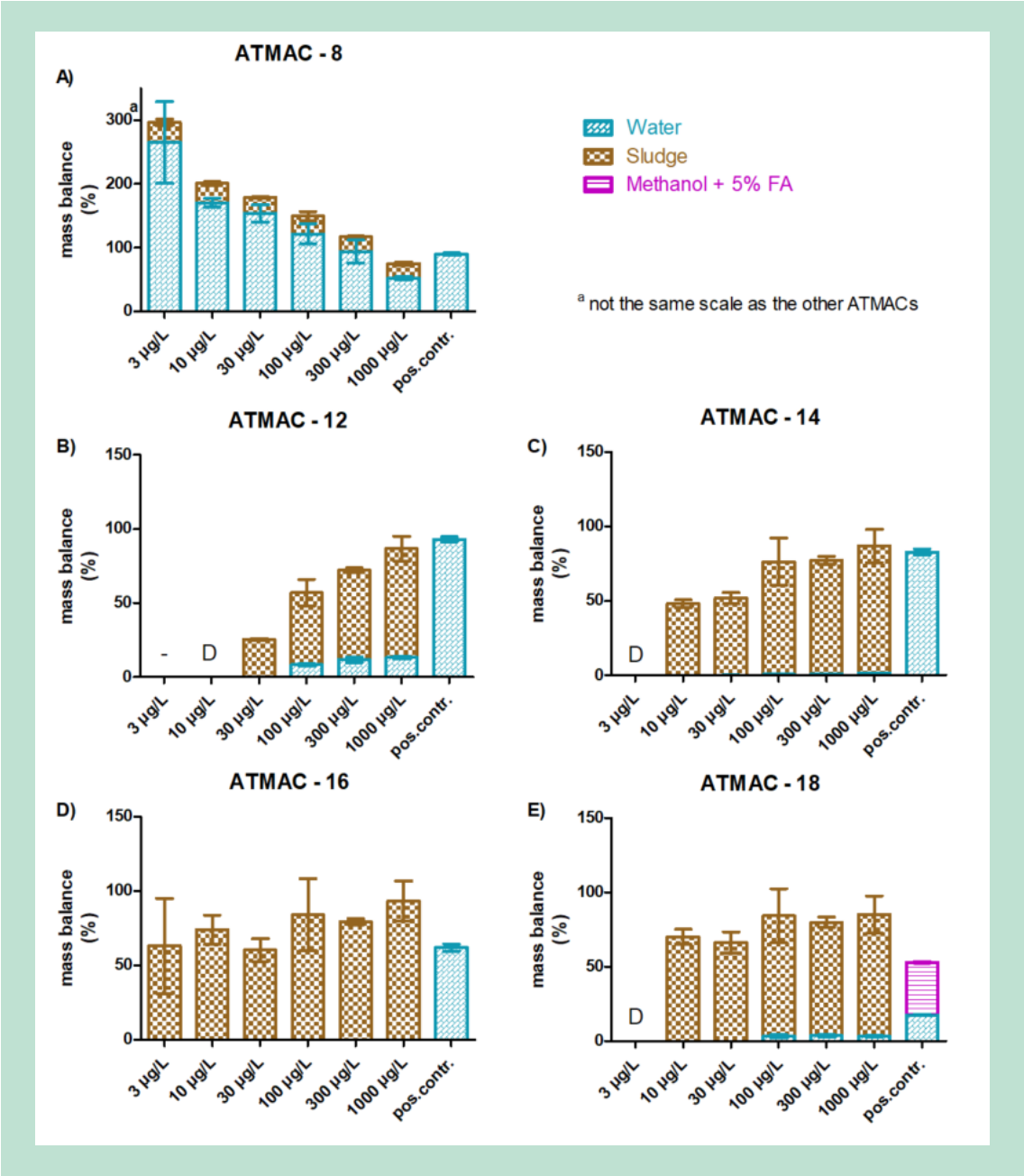


FIGURE 8.2. Exemplary raw data on partitioning of ATMACs to secondary natural sludge. The results are given in relation to the spiked amount (C/C0). If values higher than 100% are observed, the QUAT contribution from the initial sludge was considerable. Different scaling of the y axis had to be used for those compounds with considerable “natural” concentrations of the sludge.

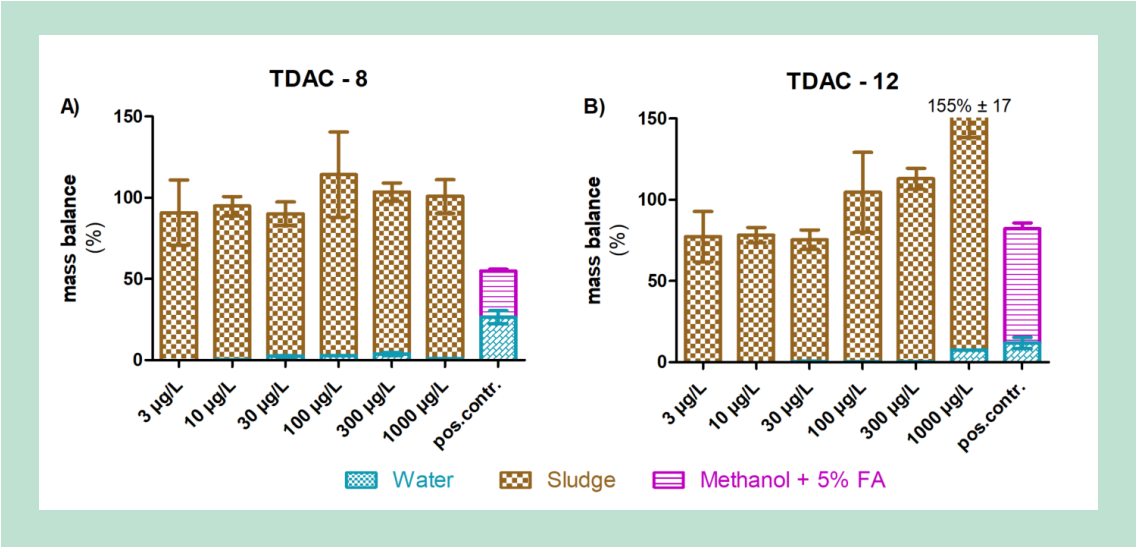


FIGURE 8.3. Exemplary raw data on partitioning of TDACs to secondary natural sludge. The results are given in relation to the spiked amount (C/C0). If values higher than 100% are observed, the TDAC contribution from the initial sludge was considerable.

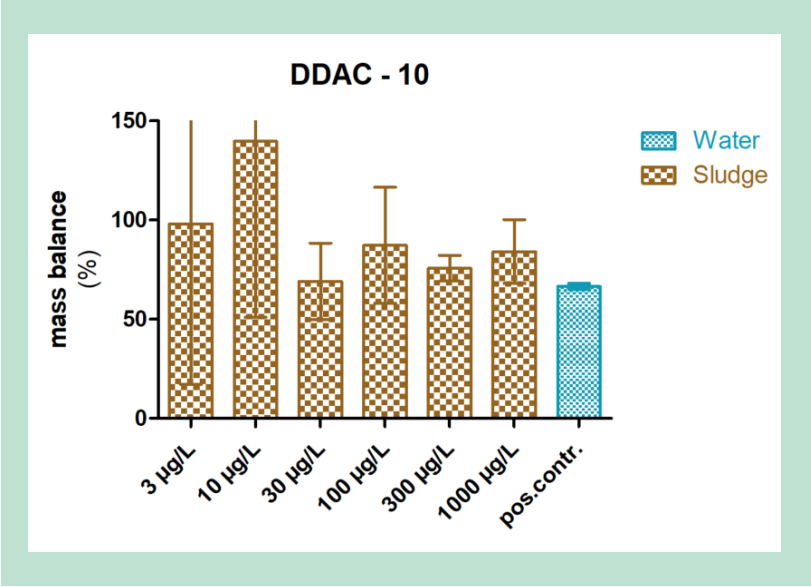


FIGURE 8.4. Exemplary raw data on partitioning of DDAC-10 to secondary natural sludge. The results are given in relation to the spiked amount (C/C0). If values higher than 100% are observed, the DDAC contribution from the initial sludge was considerable.

As lipophilicity of the compounds (K_{ow}) increase with chainlength, it is not surprising that also the partitioning to sludge changes with the chain length. The very short chainlengths are difficult to analyse and thus give incomplete mass balances (Figure 8.5)

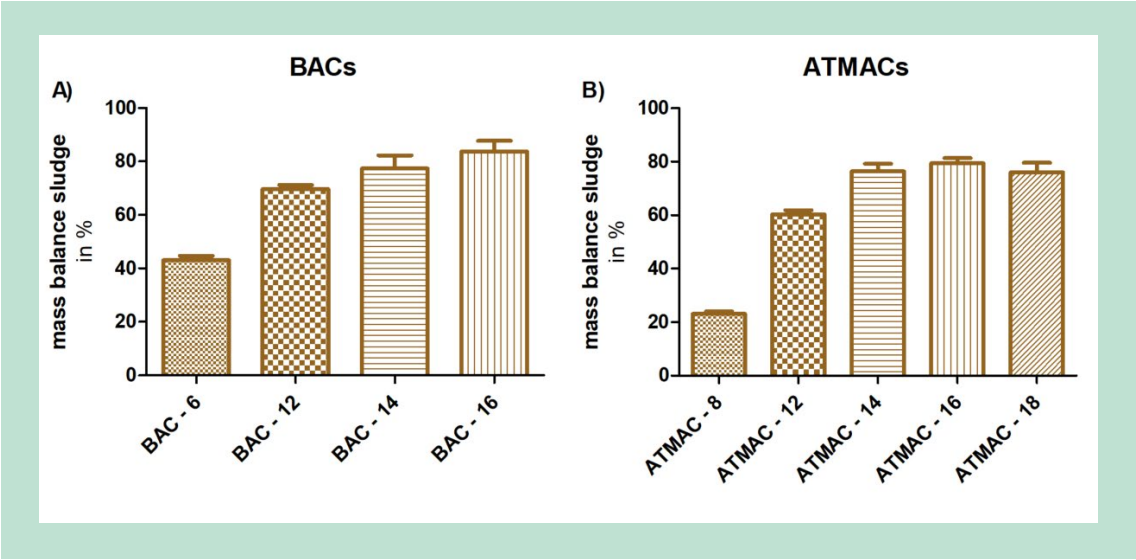


FIGURE 8.5. Influence of alkyl chain lengths on the mass balance of BACs and ATMACs in the partitioning experiments.

8.2 Biodegradation

Description of test:

For assessing the biodegradation rate constants of the QUATs in sludge, an incubation of QUATs in sludge was conducted by spiking the sludge from Bjergmarken WWTP (Roskilde, Denmark) to a concentration of 100 µg/L for each compound. The bio-incubation was performed under aerobic conditions by aerating with pressurised air (of approximately 1 L/h) (representative for BOD management and nitrification in the WWTP). To achieve sufficient data for a fit to determine reaction kinetics, 24 samples were taken from the reactors for a total time duration of 212 hours. The bio-incubation was conducted in duplicate reactors and with two positive controls (tap water spiked with QUATs). The final data treatment was performed by Graphpad Prism 9 (Dotmatics, San Diego, USA) using single first order reaction kinetics allowing for one plateau after testing for possible better fit for other kinetics. Based on R2 value, the best fitting curve for each compound was determined and the reaction rate constant was calculated from the curve equation.

Equation for single first order degradation

$$C = C_0 e^{-k_{SFO} * t} \quad \text{eq (5)}$$

Equation for single first order with a plateau

$$C = (C_0 - Plateau) * \exp(-K * X) + Plateau \quad \text{eq (6)}$$

Equation for 2 phase first order plus a plateau:

$$C = Plateau + Span_{Fast} * e^{-K_{Fast} * t} + Span_{Slow} * e^{-K_{Slow} * t} \quad \text{eq (7)}$$

Results:

To study biodegradation, a bio-incubation experiment with activated sludge and spiked QUATs was conducted over 210 hours to assess the biodegradation of QUATs in the WWTPs process. The degradation data were fitted either to single first order (eq (5)) or single first order with a plateau (equation (6) or equation for 2 phase first order with a plateau (eq (7)) (Figure 8.6 to 8.8) and Table 8.1). Degradation half-lives for QUATs ranged from 1-68 h while DDAC-18 had a degradation half-life of 434 h if fitted to first order kinetics (which is probably not adequate for this compound, opposite to all other QUATs) (Table 8.1 and Figure 8.6). TDACs and DDAC-18 were omitted from Table 8.1 as obviously something is wrong with those datasets and no reaction rate constants can be calculated for these compounds. All other compounds show very little aberration from the fitted curves.

TABLE 8.1. Single first order reaction rate constants (KSFO) of QUAT degradation in sludge with plateau and half-life $K_{1/2}$.

	BAC-6	BAC-8	BAC-10	BAC-12	BAC-14	BAC-16	BAC-18	DDAC-8	DDAC-10	DDAC-12
K_{SFO} [h^{-1}]	0.012	0.195	0.469	0.0142	0.0549	0.0102	0.0122	0.365	0.02214	0.0123
Half Life [h]	59.23	3.546	1.477	48.68	12.63	67.81	57.06	1.9	31.31	56.44
R^2	0.926	0.992	0.941	0.7617	0.8956	0.9578	0.8156	0.987	0.9178	0.8108
Y0	1.029	0.976	1.014	0.9758	1.113	1.071	0.7615	0.984	1.01	0.792
Plateau	-0.075	0.034	0.1046	0.3276	0.1362	-0.126	0.0154	0.062	0.2279	0.0323
	DDAC-14	DDAC-18	AT-MAC-8	AT-MAC-12	AT-MAC-14	AT-MAC-16	AT-MAC-18	TDAC-6	TDAC-8	TDAC-12
KSFO [h-1]	0.01598	0.0016	0.06999	0.1173	0.09431	0.02935	0.0117	0.0279	0.6899	0.01186
Half Life [h]	43.39	433.6	9.903	5.91	7.35	23.61	59.23	24.87	1.005	58.44
R2	0.8263	0.2682	0.9749	0.9883	0.9873	0.9144	0.9258	0.4665	0.7975	0.2834
Y0	1.4	0.9339	1.212	0.9454	0.9704	0.8883	1.029	0.8885	1.005	1.599
Plateau	0.00659	24.09	0.04233	0.05832	0.05743	0.1433	-0.07467	1.254	0.537	-0.8625

In Figure 8-6 to 8-8 the concentrations of QUATs during the respective degradation experiments are shown together with the fits of the equations shown in Table 8-1.

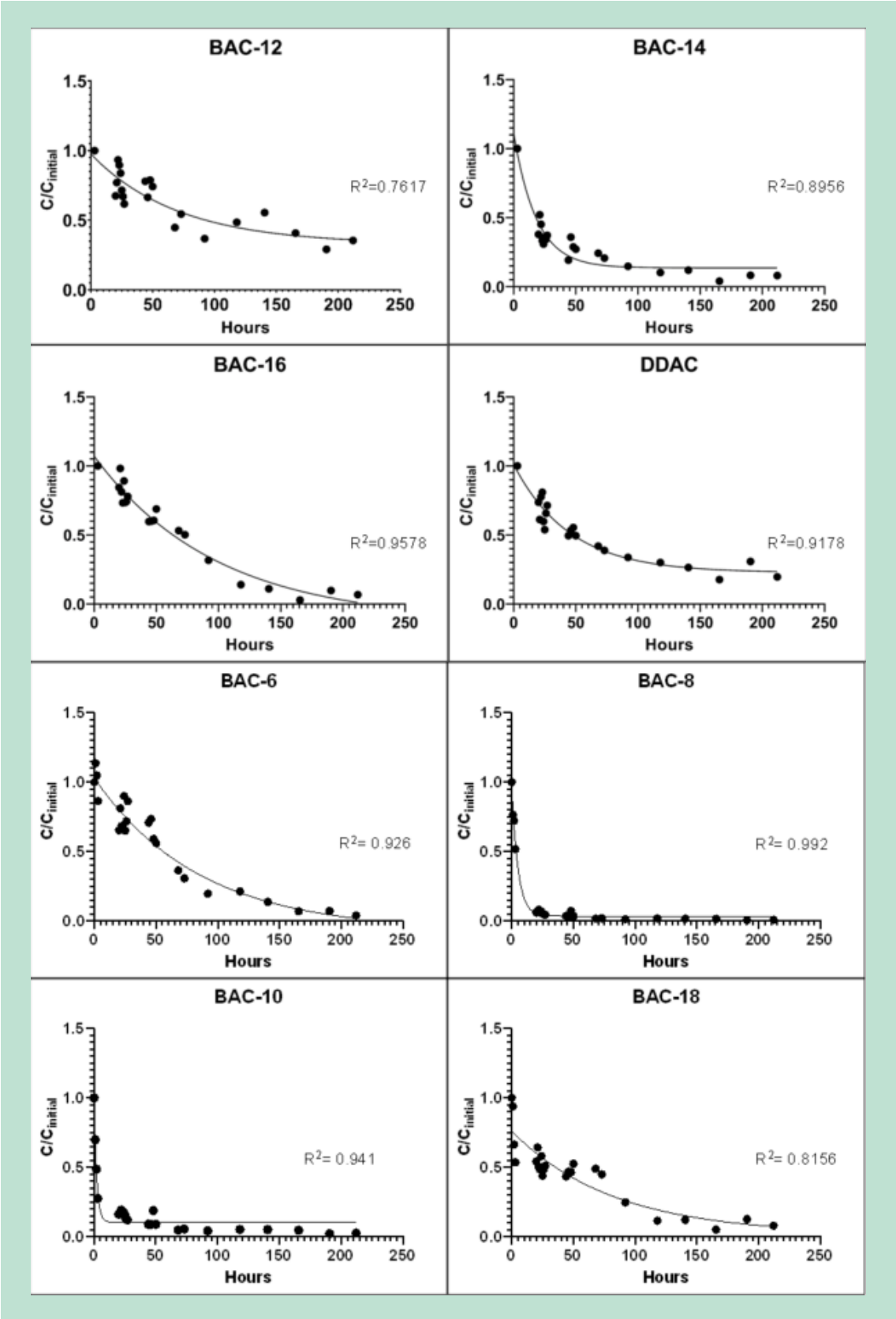


FIGURE 8.6. Reaction kinetics BACs in a sludge incubation (numerical data in Table 8.1)

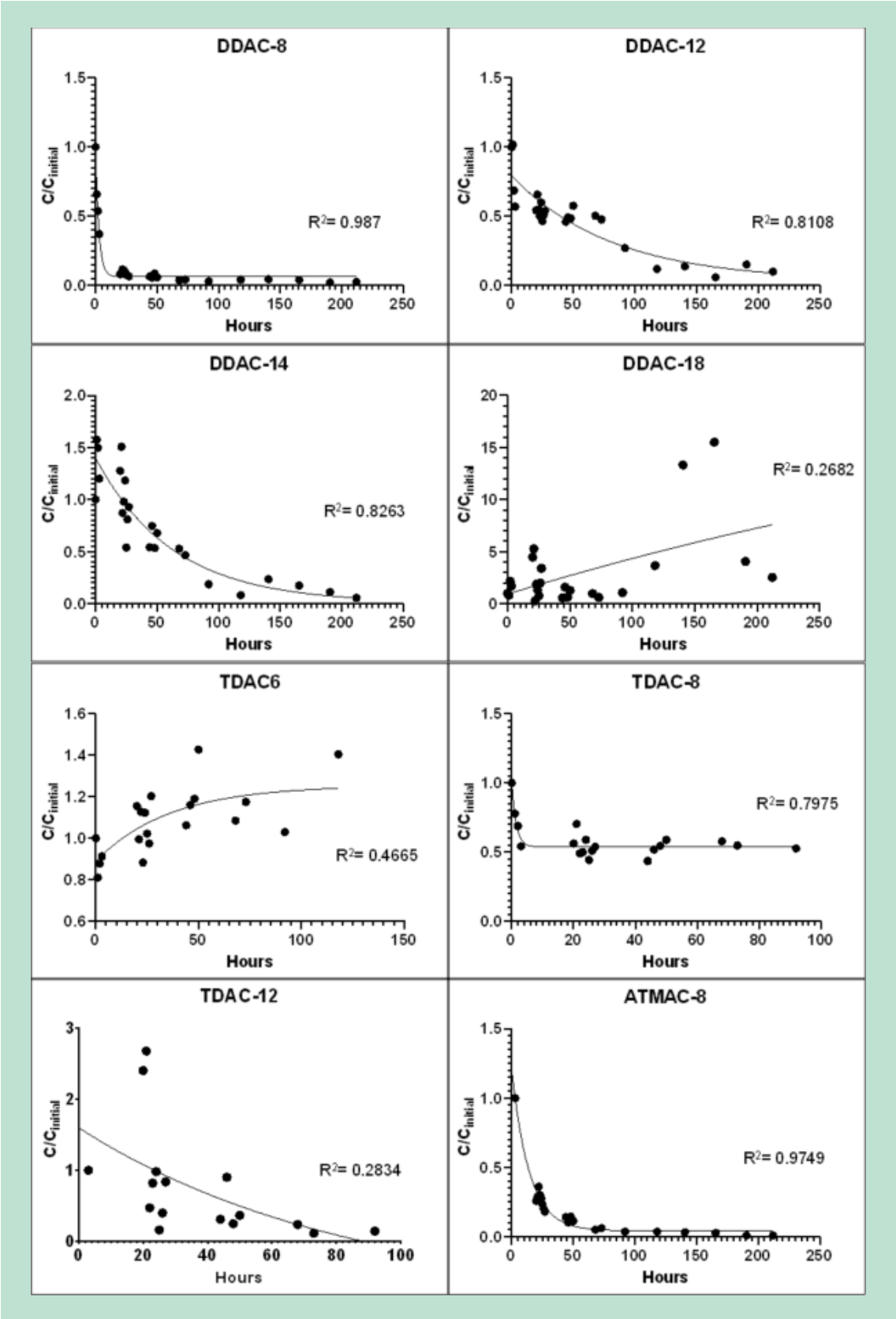


FIGURE 8.7. Reaction kinetics of selected QUATs in a sludge incubation (numerical data in Table 8.1).

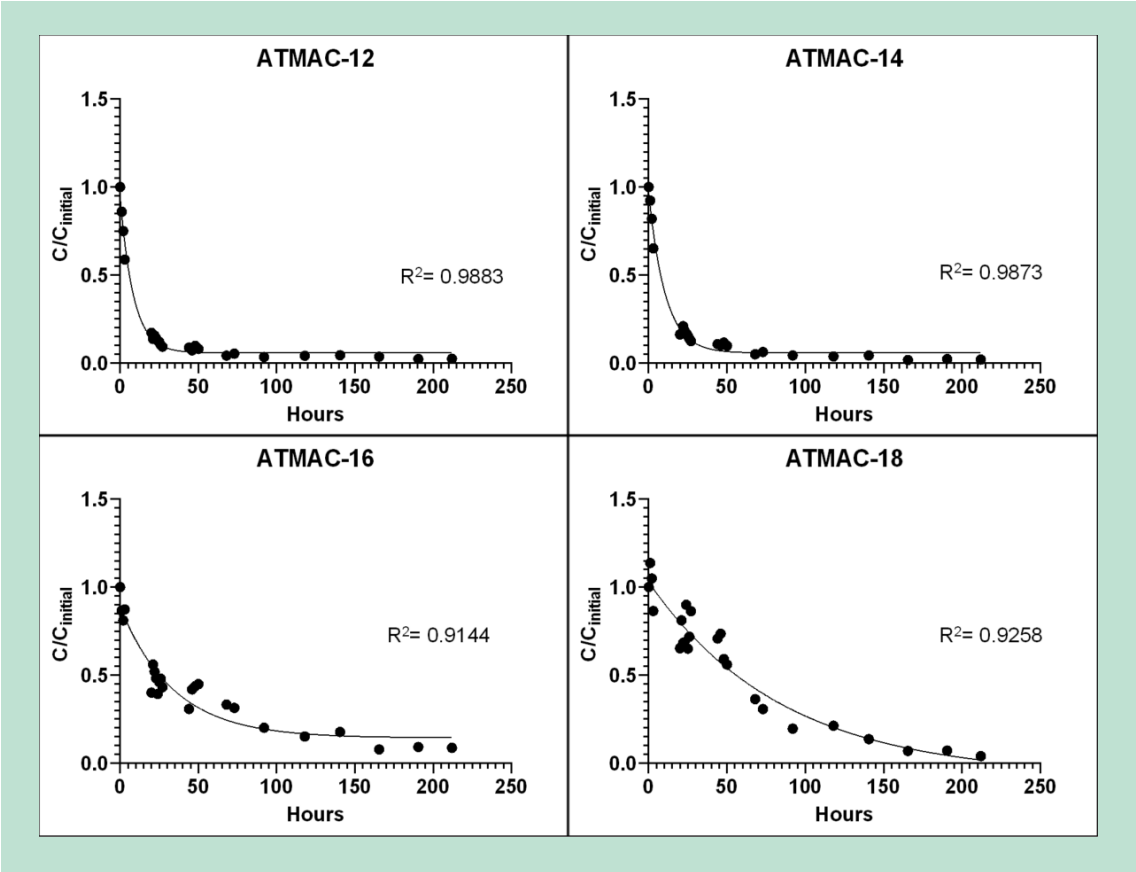


FIGURE 8.1. Reaction kinetics ATMACs in a sludge incubation (numerical data in Table 8.1).

To get from a reaction rate constant to a removal rate, the reaction time needs to be considered. The reaction time in wastewater treatment is usually considered as equal to the hydraulic retention time (assuming the residence time of the compound is the same as the residence time of the water (HRT) as the compound is quantitatively dissolved in the water). Using (eq. 5) a removal rate can be calculated with setting $C=1/2C_o$.

Even though this is the usual procedure, as most scientists take it for granted that a compound sorbed to sludge does not degrade, this approach is not necessarily quite correct and in the case of sorbing and degrading compounds the sludge retention time (SRT) becomes the better approach to assess the reaction time.

Taking a typical hydraulic residence time (HRT) of 20 h in the WWTPs into account, biodegradation is not the main contributor to the removal of QUATs. With an HRT of 20 h removal based purely on biodegradation for BAC-12, BAC-14, BAC-16 and DDAC-10 would be 20%, 55%, 16% and 27%, respectively. However, some of the treatment plants in this study had unusual high HRTs of 60 h. – This would lead to a theoretical removal of 55% for BAC-12, 80% for BAC-14, 60% of BAC-16 and 55% for DDAC-10 based on biodegradation alone. However, when there was so much partitioning on the particulate side of the system (see 3.2) that the compounds are predominantly sorbed to sludge and thus the time the compound is in the WWTP, degradation is not determined by the hydraulic retention time but by the sludge retention time - it can be argued that using the sludge retention time is more appropriate than the hydraulic retention time. Sludge retention time in CAS plants is 20-40 days (opposite to the hydraulic retention times that is 20-30 hours) so the sludge retention time is typically 10-20 times as high as the hydraulic retention time. – If that argument was true, the real removal mechanism would be a two-step

one: (rapid) partitioning followed by (slow) biodegradation. Generally speaking, the biodegradation of QUATs in WWTPs could be substantial, but the details will depend on both the details of the design of the WWTP and the respective QUAT congener. In the framework of this experiment, it was important to show (for the first time) not only sorption but also that biodegradation is significantly involved in the removal of these compounds.

8.3 Removal in WWTPs

Concentrations of QUAT compounds in primary sludge ranged from 3.000 (BAC-16) to 144.000 µg/kg, i.e., 3-144 mg/Kg (DDAC-10) and the pattern was similar to the one in the influent water (Table 8.2). Highest concentrations were found for DDAC-10, ranging from 84.000 to 144.000 µg/kg, followed by BAC-12 (29.000 to 49.000 µg/kg), BAC-14 (13.000 to 28.000 µg/kg) and BAC-16 (3.000 to 4.000 µg/kg). These values are in the same range as those determined by Kaj et al. (2014) who reported concentrations from 5.000 ng/g to 100.000 ng/g. While these concentrations are high, especially in relation to fish toxicity, they do not reach levels that are suggested to hinder BOD or nitrogen removal in the WWTP (Conidi et al., 2019).

Based on the measured data it is possible to calculate removal in the WWTPs for each compound on a daily basis (Table 8.2). Even though concentrations vary considerably from day to day in one WWTP and from WWTP to WWTP, the removal rates calculated on a daily basis are quite reproducible for each compound (Table 8.2). Removal in all WWTPs is relatively high for all the QUATs and ranges (compound specific) from 86 and >99 %. The lowest removal was detected for BAC 14 on single days in two WWTPs while other removals were usually exceeding 97%. These removal data are consistent with data presented in literature (Clara et al., 2007).

In the outlet of the WWTPs five metabolites from BACs were detected, TP208B, TP152, TP250, N,N-Dimethylbenzylamine and N,N-Dimethyldecyclamine. These findings further support that biodegradation is significantly involved in the removal of QUATs.

TABLE 8.2. Concentrations and removal of main QUATs in the WWTPs.

	BAC-12		BAC-14		BAC-16		DDAC	
Median	Concentration	Removal	Concentration	Removal	Concentration	Removal	Concentration	Removal (%)
	[ng/L]	[ng/L]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]
WWTP Måløv IN	1830	-98	68	-92	168	-99	3118	-100
WWTP Måløv OUT	35		5		1		0	
WWTP Stenløse IN	1122	-99	40	-97	125	-100	1788	-100
WWTP Stenløse OUT	15		1		0		0	

WWTP Ølstykke IN	1072	-97	43	-81	117	-100	1728	-100
WWTP Ølstykke OUT	31		8		0		0	

8.4 Discussions on QUATs in WWTPs

The concentration in the WWTP we determined as dissolved in raw wastewater is one order of magnitude lower than those described in the older literature (Clara et al., 2007). However, in that study the total QUATs (sorbed to primary sludge and dissolved) were reported. If the data from primary sludge and dissolved in water from our study is combined, the obtained results are in the same order of magnitude as the older studies.

Östman et al., 2018 did mass balance experiments on three Swedish WWTPs. These authors also report high removal rates exceeding 99% for BAC-12, BAC-14 and DDAC. They report about 50% sorbed to sludge, but do not discuss the mechanisms behind the gap in detail. In comparison to this our new study reveals that the missing gap in this dataset, most probably is biodegradation of the respective compounds.

Contrary to conventional beliefs or commonly held notions, the QUATs can be degraded while being sorbed to sludge (all data on figure 8.1 is based on processes and concentrations in sludge). To find a relevant timing, this needs to be linked not to the hydraulic retention time (10-60 h), but to the sludge retention time (10-30 d). Thus, in the overall picture, WWTPs are able to biodegrade a considerable fraction of the incoming QUATs.

However, as all sludge is recirculated and thus repeatedly exposed to fresh wastewater on multiple occasions., there will not be a sludge with low QUAT concentrations, except if it undergoes a separated aerobic treatment, which is not implemented in any Danish WWTP resulting in the mass balance as pointed out by Östman et al., 2018 (as discussed above).

8.5 Key findings on QUATs in WWTPs

- The WWTP inlet concentrations are high (exceeding 1000 ng/L) in the dissolved fraction.
- BAC-12, BAC-14, BAC-16 and DDAC-10 dominated the raw wastewater.
- The concentrations in the sludge are very high and are typically ranging from 3.000 to 144.000 ng/g (3-144 mg/Kg).
- Removal rates of these compounds in WWTPs are considerable and usually exceeding 99%, which is in agreement with the newer assessment documents of BAC and DDAC.
- Concentrations in the WWTP effluents are thus usually between 1 and 20 ng/L.
- The most likely removal mechanism is the rapid sorption of the compounds to the sludge, which is subsequently followed by a slow process of biodegradation. Concerning WWTPs, the main emissions will occur with the sludge.

9. Monitoring in surface water (WP5)

Monitoring in surface waters was conducted in two phases: a) as classical monitoring to establish a baseline with low resolution but high distribution b) as process study with high resolution and low distribution.
All in all, 7 BAC isomers, 5 DDAC isomers, 5 ATMAC isomers and 3 TDAC isomers were quantified (Table 3.3). However, with a few exceptions only BAC-12, BAC-14 ABC-16 and DDAC-10 were detected in the samples.

Monitoring:
Sampling campaigns performed in Havelse Å and Gudenå rivers have been conducted during dry weather, focus on dry weather contributions such as WWTPs without emissions of QUATs through runoff/stormwater and flied leaching.
The results of the sampling campaign carried out on the Gudenå river show levels of contamination below limit of detection, despite being affected by 7 different WWTPs. These results were similar with what has been reported by the National Monitoring Program (Novana) in the summer campaign, where they found concentrations of BACs below or close to the limit of detection.
As the Havelse Å is not affected by WWTPs and has a mainly agricultural/rural, sparsely populated catchment area, we did not expect to find high concentrations of QUATs. However, we detected high quantities of QUATs with 29 ug/L BAC-12 and 7 ug/L BAC-14 (Table 9.1) in a small tributary of the river. The surrounding area is characterized by cultivated fields and grazing cows and this could indicate that the contamination detected may be due the use of products containing QUATs in agriculture and veterinary hygiene.
Taken together, these results do not clearly indicate the origin of QUATs in surface waters.
To better understand if the weather and the WWTPs have an impact on the QUATs found in surface water we have monitored the stream Værebro Å (affected by 3 WWTPs) during one year in dry and wet conditions.

TABLE 9.1a. Concentrations of QUATs in Havelse Å

Catchment area (date)	Concentrations in surface water (µg/L)	BAC-12	BAC-14	BAC-16	DDAC-10
Havelse Å (12/11/21)	Havelse Å spring	< 0.001	< 0.001	< 0.001	< 0.001
Havelse Å (12/11/21)	Kellerød Å (Tributary)	< 0.001	< 0.001	< 0.001	< 0.001
Havelse Å (12/11/21)	Havelse Å After confluent	< 0.001	< 0.001	< 0.001	< 0.001
Havelse Å (12/11/21)	Havelse Å/Gørløse Å (Tributary)	29,84 ± 4.50	7,06 ± 0.94	0,07 ± 0.01	0,22 ± 0.04
Havelse Å (12/11/21)	Havelse Å after confluent (Gørløse)	0,60 ± 0.02	0,13 ± 0.02	< 0.001	< 0.001

Havelse Å (12/11/21)	Havelse Å mouth	0,19 ± 0.08	0,06 ± 0.01	< 0.001	< 0.001
Havelse Å (26/11/21)	Kellerød Å (Tributary)	< 0.001	< 0.001	< 0.001	< 0.001
Havelse Å (26/11/21)	Havelse Å/Gørløse Å (Tributary)	< 0.001	< 0.001	< 0.001	< 0.001
Havelse Å (26/11/21)	Havelse Å after confluent (Gørløse)	< 0.001	< 0.001	< 0.001	< 0.001
Havelse Å (26/07/22)	New tibutary	< 0.001	< 0.001	< 0.001	< 0.001
Havelse Å (26/07/22)	Havelse Å Before Kellerød tributary	< 0.001	< 0.001	< 0.001	< 0.001
Havelse Å (26/07/22)	Kellerød Å (Tributary)	< 0.001	< 0.001	< 0.001	< 0.001
Havelse Å (26/07/22)	Havelse Å after Kellerød tributary	< 0.001	< 0.001	< 0.001	< 0.001
Havelse Å (26/07/22)	Havelse Å mouth	< 0.001	< 0.001	< 0.001	< 0.001

TABLE 9.2a. Concentrations of QUATs in Gudenå

Gudenå 07-08/04/22)	**Gudenå spring**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Gudenå 07-08/04/22)	After Torring´s town	< 0.001	< 0.001	< 0.001	< 0.001
Gudenå 07-08/04/22)	Uspstrem WWTP1 (Åle)	< 0.001	< 0.001	< 0.001	< 0.001
Gudenå 07-08/04/22)	Dowstream WWTP1 (Åle)	< 0.001	< 0.001	< 0.001	< 0.001
Gudenå 07-08/04/22)	Matterup Å (tributary)	< 0.001	< 0.001	< 0.001	< 0.001
Gudenå 07-08/04/22)	Ustream WWTP2 (Brædstrup)	< 0.001	< 0.001	< 0.001	< 0.001
Gudenå 07-08/04/22)	Dowstream WWTP2 (Brædstrup)	< 0.001	< 0.001	< 0.001	< 0.001
Gudenå 07-08/04/22)	Salten langsø (tributary)	< 0.001	< 0.001	< 0.001	< 0.001
Gudenå 07-08/04/22)	Emborg Bro (before Mossø)	< 0.001	< 0.001	< 0.001	< 0.001
Gudenå 07-08/04/22)	Emborg Bro (after Mossø)	< 0.001	< 0.001	< 0.001	< 0.001
Gudenå 07-08/04/22)	Alling Vest Teltplads (before julsø)	< 0.001	< 0.001	< 0.001	< 0.001
Gudenå 07-08/04/22)	After Julsø	< 0.001	< 0.001	< 0.001	< 0.001
Gudenå 07-08/04/22)	Uspstrem WWTP3 (Silkeborg)	< 0.001	< 0.001	< 0.001	< 0.001
Gudenå 07-08/04/22)	Dowstream WWTP3 (Silkeborg)	< 0.001	< 0.001	< 0.001	< 0.001
Gudenå 07-08/04/22)	Uspstrem WWTP4 (Truust)	< 0.001	< 0.001	< 0.001	< 0.001

Gudenå 07-08/04/22)	Dowstream WWTP4 (Truust)	< 0.001	< 0.001	< 0.001	< 0.001
Gudenå 07-08/04/22)	Tange Å (tributary)	< 0.001	< 0.001	< 0.001	< 0.001
Gudenå 07-08/04/22)	Uspstrem WWTP5 (Bjerringbro)	< 0.001	< 0.001	< 0.001	< 0.001
Gudenå 07-08/04/22)	Dowstream WWTP5 (Bjerringbro)	< 0.001	< 0.001	< 0.001	< 0.001
Gudenå 07-08/04/22)	Uspstrem WWTP6 (Ulstrup)	< 0.001	< 0.001	< 0.001	< 0.001
Gudenå 07-08/04/22)	Dowstream WWTP6 (Ulstrup)	< 0.001	< 0.001	< 0.001	< 0.001
Gudenå 07-08/04/22)	Uspstrem WWTP7 (Langå Hundeskov)	< 0.001	< 0.001	< 0.001	< 0.001
Gudenå 07-08/04/22)	Dowstream WWTP7 (Langå Hundeskov)	< 0.001	< 0.001	< 0.001	< 0.001
Gudenå 07-08/04/22)	Norre Å (tributary)	< 0.001	< 0.001	< 0.001	< 0.001
Gudenå 07-08/04/22)	Mouth (Randers)	< 0.001	< 0.001	< 0.001	< 0.001

Process study in Værebro Å in dry and wet weather

This study was conducted to enable determination of sources and processes that lead to high concentrations in surface waters as well as to compare concentrations over time.
The study was conducted following two hypotheses.

1. The wastewater treatment plants are the main source of QUATs in the recipients.
2. Urban water runoff (of treated roofs, terraces etc.) is the main source of QUATs in the recipients.

Since predominantly three QUATs (BAC-12, BAC-14, and DDAC-10) were detected, the discussion on the concentrations and the research of the origin from the emission into the environment is focused on these compounds. Metoprolol is used as a wastewater marker, as it is one of the most commonly used blood pressure regulators and thus one of the most frequently used pharmaceuticals in Denmark (Kisielius, et al., 2024). It is not removed in wastewater treatment or underlying environmental processes that significantly impacts its mass flows.
An overview of the concentration of the main QUATs and metoprolol are shown in Figure 9.1 to 9.8.

Rainy weather:
Highest concentrations were found during (and following) a rainy period (in October 2021 Figure 9.2). According to the Danish Meteorological Institute the area of the sampling was affected by 8.7 mm of rainwater during the 24 hours before the sampling and 1.2 mm of rainwater occurred during the active sampling activities at the Værebro Å.
The major contribution of QUATs was detected in a small tributary (Bunds Å, station 3, Figure 9.1 Figure 3.6) with concentrations of 81 ng/L of BAC-12 and 57 ng/L of BAC-14. This tributary is in a rural/agriculture area and is, to our knowledge, not affected from any WWTPs. Therefore,

the pollution found in this tributary could be related either i) to the direct use of products containing QUATs in agriculture and/or ii) to the leaching of surfaces previously treated with QUATs (Gromarie et al. 2015, Bressy et al. 2012, Bollmann et al. 2016).

All determined concentrations are well below the EC_{50} (fish) between 5.9 and 280 µg/L (USEPA, 2006)). However, Bunds Å, station 3 is much closer to the PNECwater of 400 ng/L for "Alkyl (C12-16) dimethylbenzyl ammonium chloride" (derived from chronic data on three trophic levels and an assessment factor of 10 – daphnia and algae being the most sensitive species). Also, risk quotients are consistently calculated to be higher in the sediment than in the water. Data on sediment dwelling organisms show that the effect level is not very far from the one calculated with EPM.

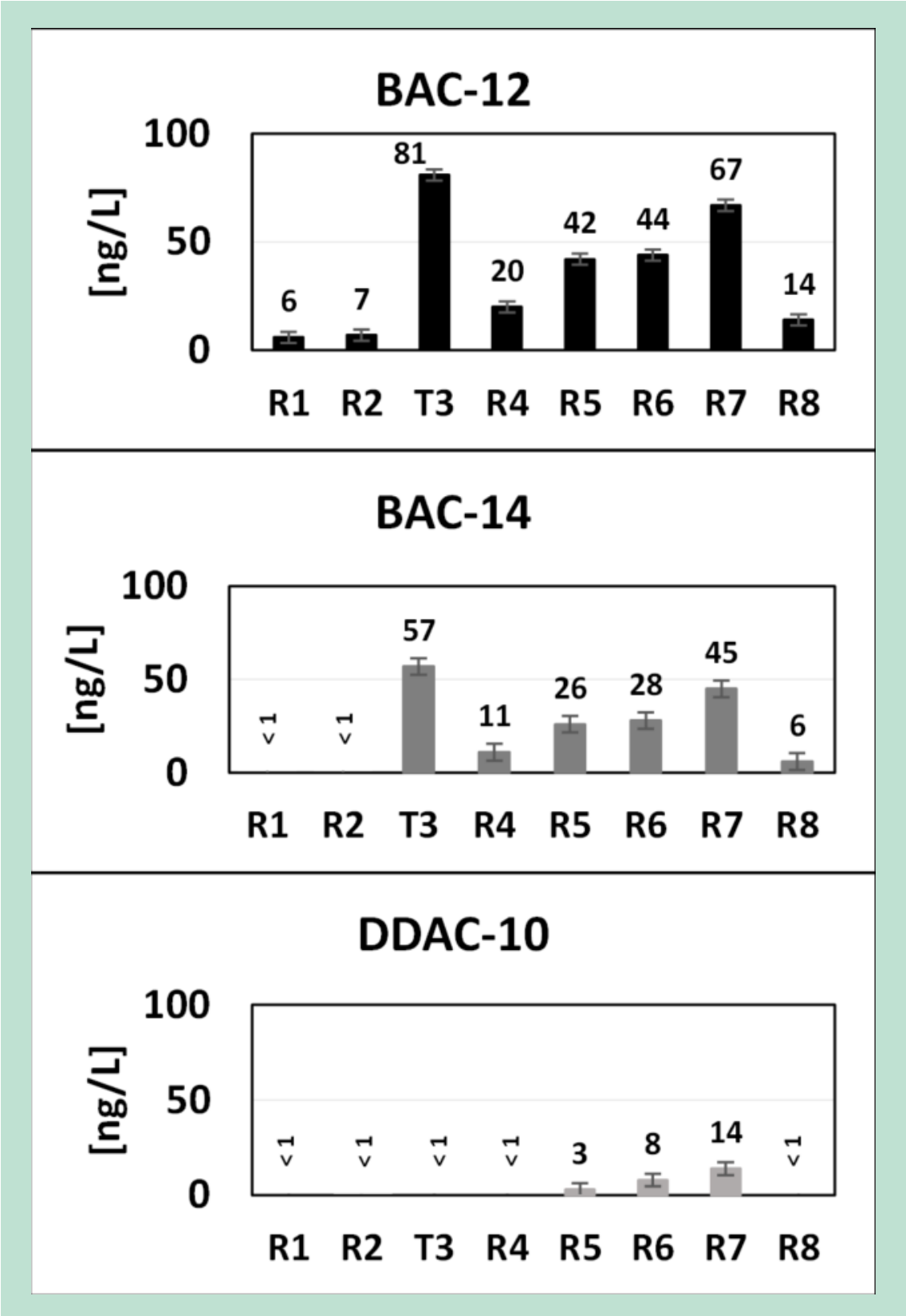


FIGURE 9.1. Concentration of QUATs along the Værebro Å river during rainy weather (last 24 h: 8.7 mm; last 4h 1.2 mm) in October 2021.

Following the course of the river the concentrations of QUATs during wet weather (Figure 9-1) increase from Måløv (1) to the most downstream sampling station (8) and decrease significantly

in the last sampling station (mouth of the river). This sampling station is very close to the sea and mixing between river and with marine waters could be the cause of the decrease of concentration.
The data from this experiment support the hypothesis that the runoff from treated urban areas give a major contribution to the QUAT loads in the recipients.

Dry weather

During dry weather conditions all QUAT concentrations were below the limit of detection, as shown in the Figure 9-2. This is in spite of WWTPs discharge into Værebro Å with concentrations between 1 and 60 ng/L.

Sampling during dry weather, following rain

During the sampling campaigns which were performed succeeding two rain events, BAC-12 and BAC-14 can only be determined in a few locations (around Måløv town) (Figure 9-3 Figure 9-4).

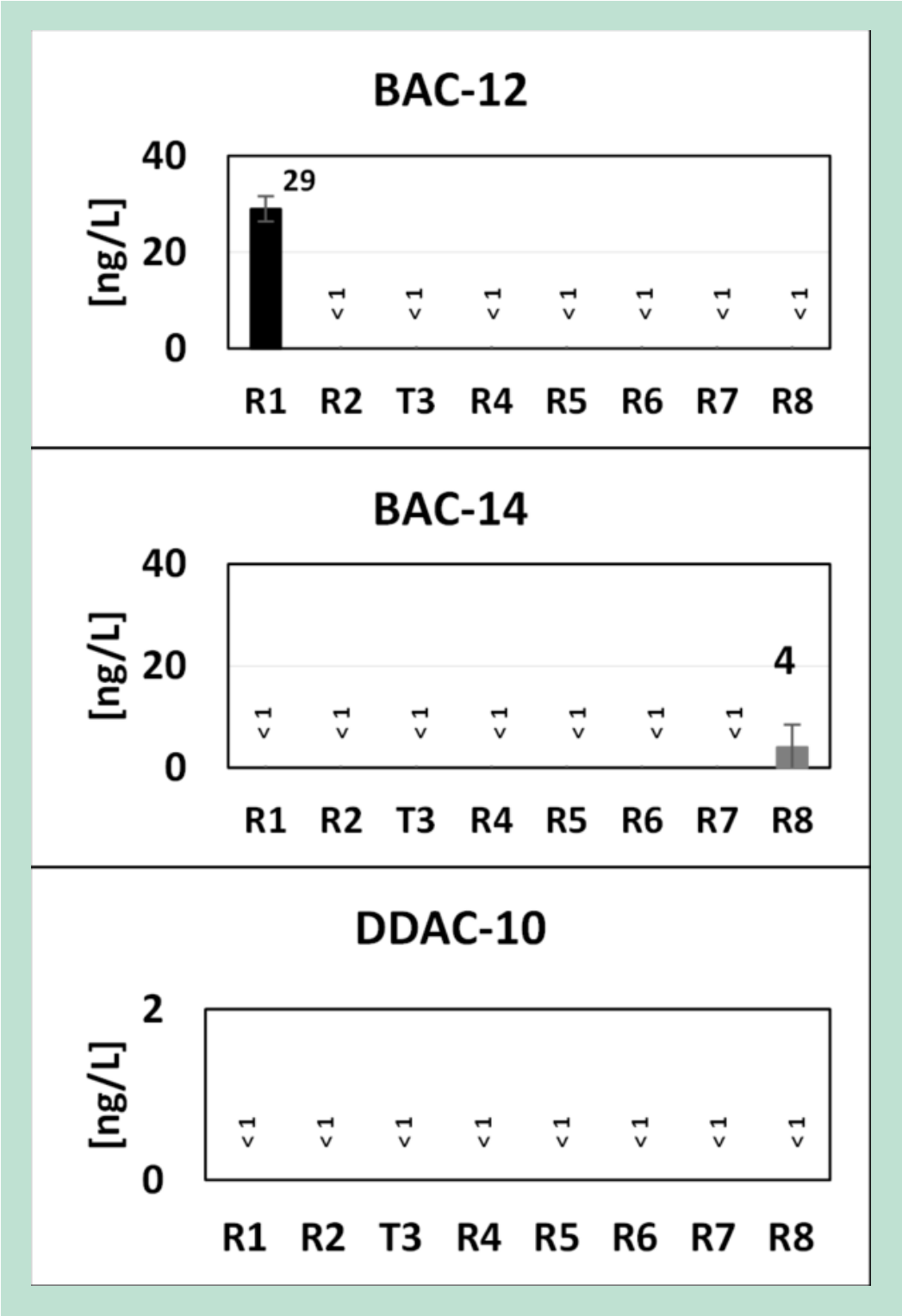


FIGURE 9.2. Concentration of QUATs along the Værebro Å river during dry weather (0 mm rain during the last 24 h) in March 2022.

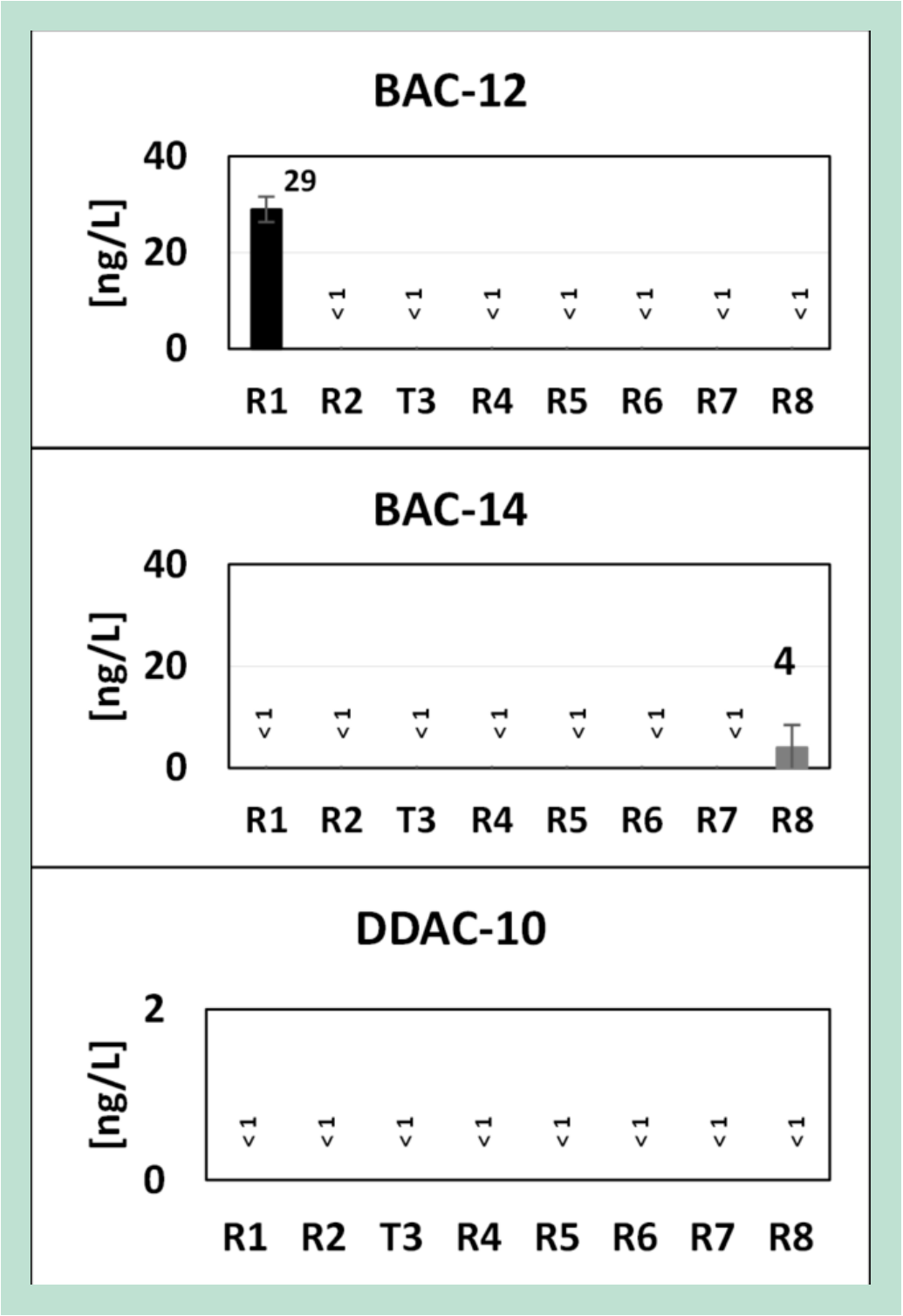


FIGURE 9.3. Concentration of QUATs along the Værebro Å river during a dry period after a rain event (11 mm in the 24 h and 0 mm in the 4 h preceding the sampling) in May 2022.

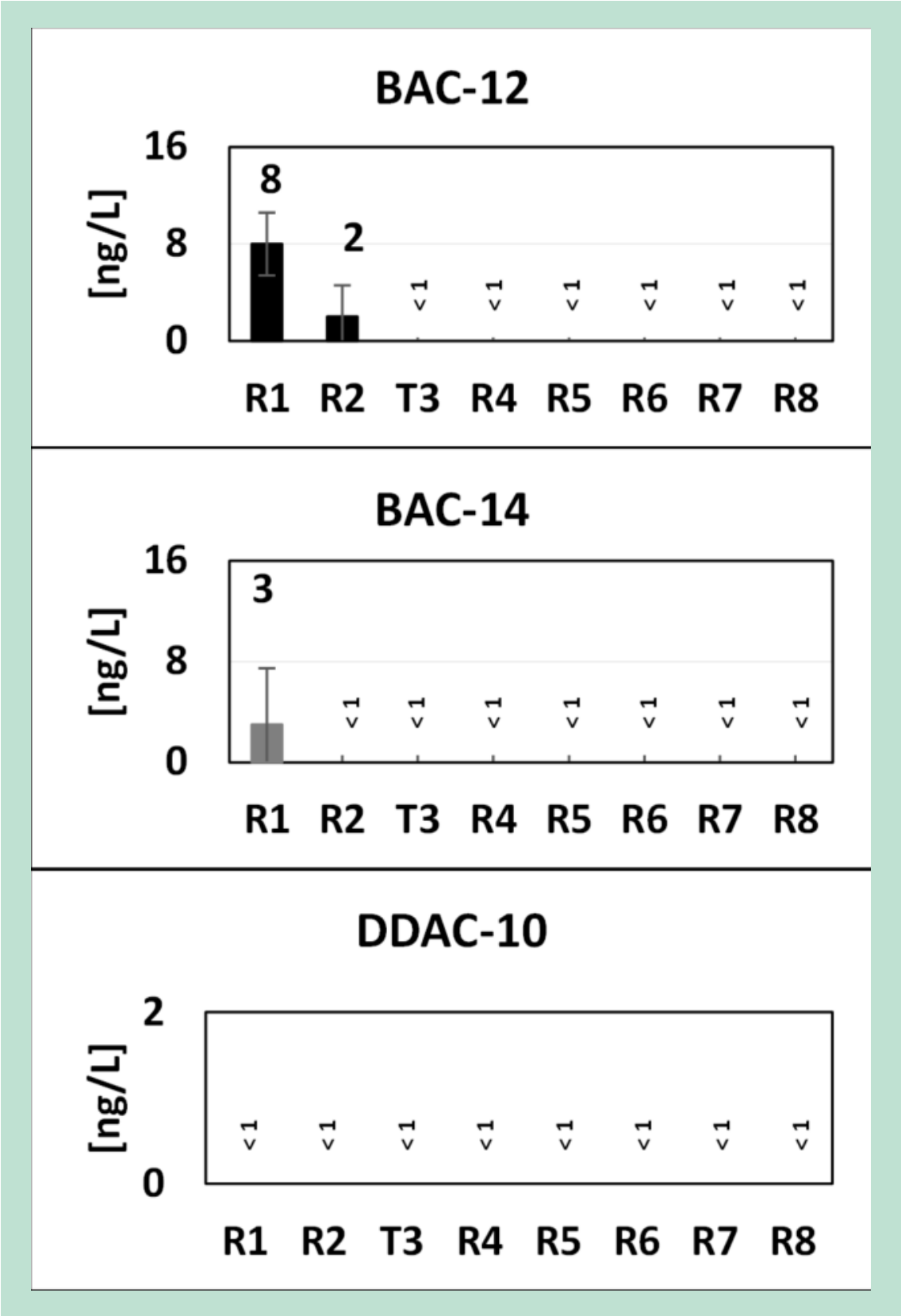


FIGURE 9.4. Concentrations along the Værebro Å river during a relative dry period (0.3 mm the last 4 h preceding sampling) after a small rain event (5.4 mm in the 24 h preced-ing the sampling) in July 2022.

If a significant fraction of the emissions were related to WWTPs discharge, we should find QUATs levels more constant over the time and not only during rain events as is the case for metoprolol (Figure 9-5 to Figure 9-8).

If wastewater treatment plants were relevant sources for QUATs in surface waters, elevated QUAT concentrations were expected at station 2 (downstream Måløv WWTP), 6 (downstream Stenløse WWTP) and 7 (downstream Ølstykke WWTP) would have been expected. This dataset falsifies the hypothesis that QUAT discharge of WWTPs contributes significantly to the loading of the recipients.

Comparison of QUAT concentrations to a conservative wastewater tracer.

As a comparison, metoprolol is used. – Metoprolol is singly used as blood pressure regulator and its presence in the environment is due to this compound is excreted unchanged and thus most of the used metoprolol is transported with the wastewater to the wastewater treatment plants. In the WWTPs, only a small fraction (10%) of metoprolol is removed as it does not sorb very well, nor is it easily biodegraded (Wick et al., 2009). Typical metoprolol WWTP effluent concentrations are around 2 µg/L, which is also the case in the WWTPs discharging into Værebro Å (Table 9.3).
The samples that were expected not to contain wastewater (such as samples 1 and 3 (Figure 9.5)) consistently showed no detectable levels of metoprolol (below 0.05 µg/L). Though some variability between the datasets were detected, no systematic changes were visible.

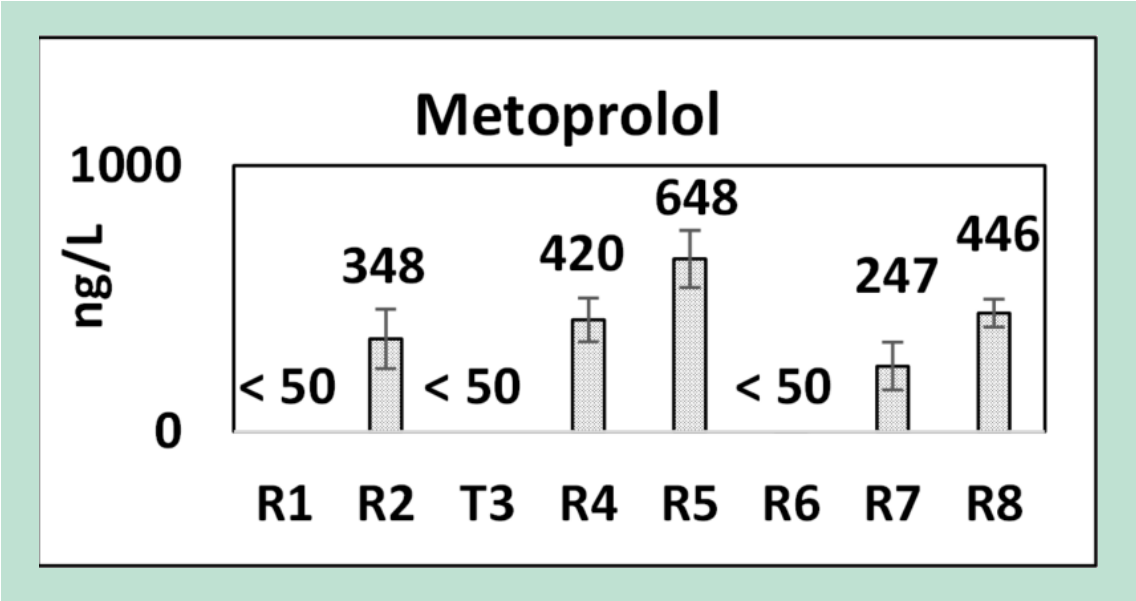


FIGURE 9.5. Concentrations of metoprolol (ng/L) in Værebro Å river in October 2021.

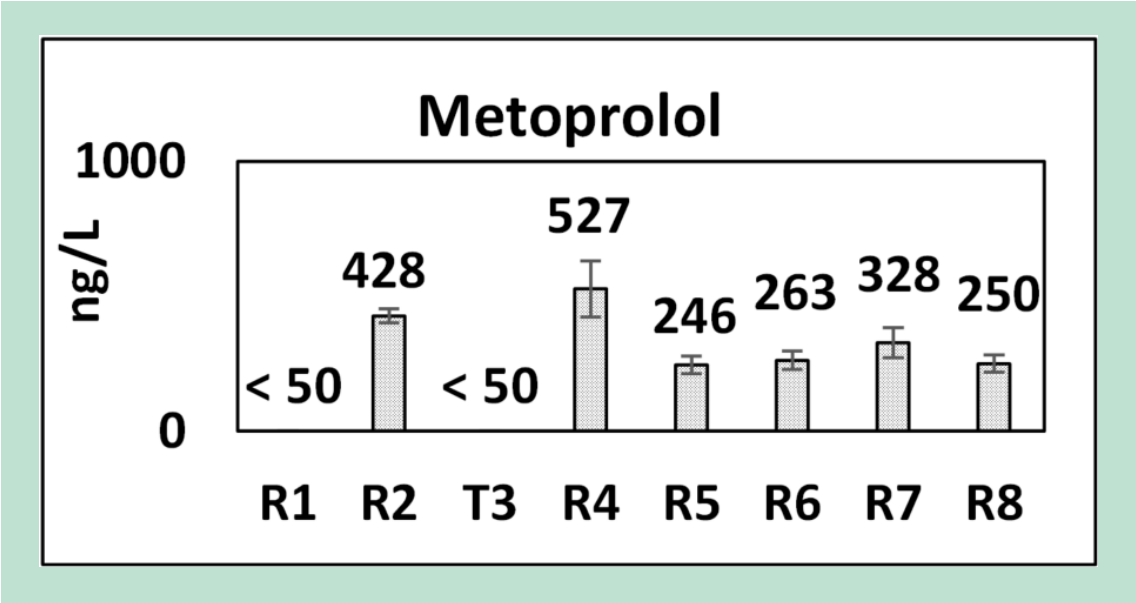


FIGURE 9.6. Concentrations of metoprolol (ng/L) in Værebro Å river in March 2022.

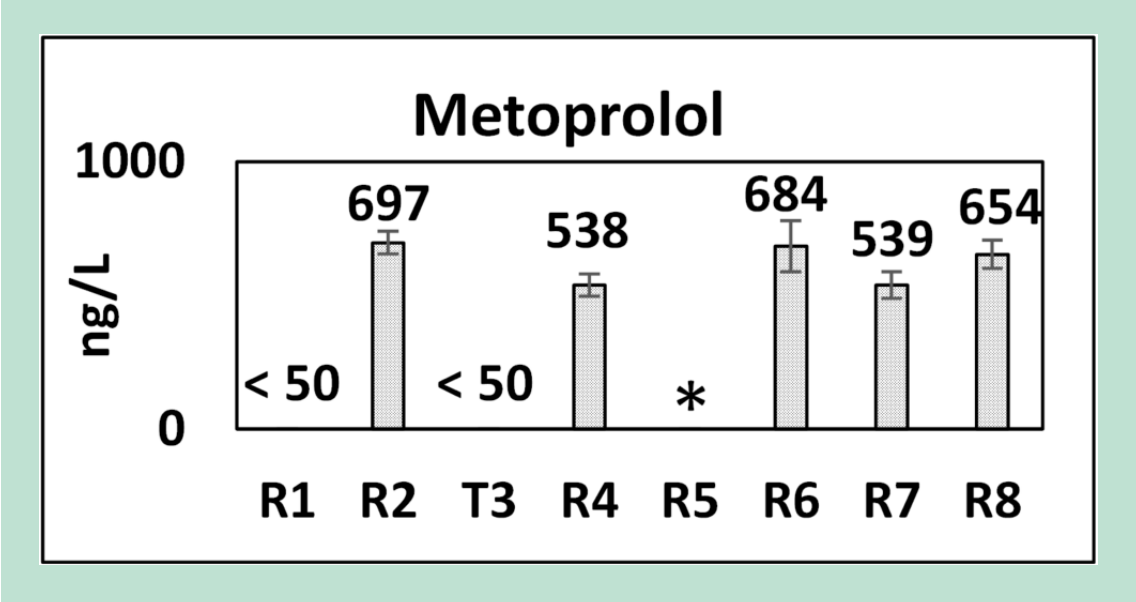


FIGURE 9.7. Concentrations of metoprolol (ng/L) in Værebro Å river in May 2022 (* lost sample for this quantification).

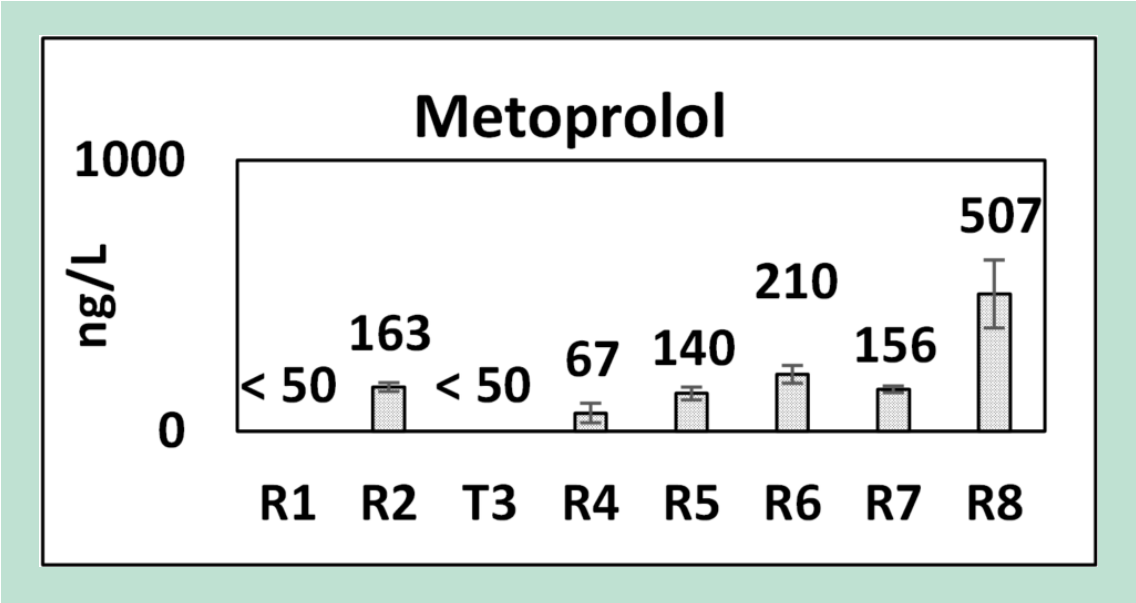


FIGURE 9.8. Concentrations of metoprolol (ng/L) in Værebro Å river in July 2022.

Wastewater treatment plants effluents

To test whether the WWTPs are the main source of QUATs in the Værebro Å, we measured the discharge concentrations of QUATs in the effluents of the three WWTPs along the river. The results (Table 9.3) show BAC-12 is the predominate QUAT in the effluents with values between 15 and 35 ng/L, followed by BAC-14 from 3 to 9 ng/L and BAC-16 with 1 ng/L. DDAC-10 was not detected in the effluents. These concentrations in WWTP effluents are below the wet weather concentrations in the creek, where a maximum concentration of 67 ng/L was found (station 7, Figure 9.1). On the other hand, the WWTP effluent concentrations are considerably above the dry weather concentrations in the creek, where all QUAT concentrations were below LOD. Taking the metoprolol values into account, the average dilution of WWTP effluents in this river is factor 12 (calculated as WWTP effluent versus the nearest downstream sampling station), i.e., the river thus contains relative consistently 8% treated wastewater. This percentage can be decreased by rain or increase of groundwater inflow.

Thus, in dry weather there was a considerable decline of QUAT concentrations (from discharge to first sample station) under dry weather as the measured concentrations were almost always below LOD in dry weather. Recent studies observed high sorption constants of QUATs to both sludges (data from this report) and clays (Montmorillonite (Zanini et al., 2013)), hence we assume that the QUATs discharged by WWTP are sorbed rather rapidly to river sediments. Further research on concentrations in sediments and solids is needed to confirm this hypothesis.

In wet weather conditions on the other hand, we assume that a) massive sources due to surface runoff and b) elevated flow velocities led to the observed concentrations.

The hypothesis that runoff from urban and agriculture plays an important role is further cemented by the estimated BAC-12 and BAC-14 concentrations during dry weather in the stream which are considerably lower than the concentrations measured in the wet-weather event in October.

TABLE 9.3. Average of QUATs and metoprolol concentrations in the effluents of the WWTPs.

WWTP	BAC-12 (ng/L)	BAC-14 (ng/L)	BAC-16 (ng/L)	Metoprolol (µg/L)
Måløv	35	6	1	2.52
Stenløse	15	3	1	1.71
Ølstykke	31	9	1	3.76

9.1 Key findings on QUATs in surface waters

1. QUAT concentrations vary due to weather conditions with higher concentrations found during rain events.
2. Even though QUAT concentrations in WWTPs effluents can be determined, these emissions have minimal impact in small rivers like the Værebro Å as they are either sorbed or degraded rapidly.
3. There are clear indications that emissions due to urban runoff control the concentrations in small rivers.

FIGURE 9.9 Professional cleaning a roof in a building complex (use of biocides during this individual operation is unknown).

10. Conclusions

The project succeeded in establishing methods for the elucidation of QUATs fate into the environment.

Several phototransformation- and biotransformation products were characterized and tentatively added to the analysis.

At the beginning of the project, QUATs were prevalent in human hygiene products as well as terrace and roof cleaning products. Presence of QUATs in roof and terrace cleaning products have decreased during the project as they were replaced by pelargonic acid in the products.

High concentrations of QUATs were determined in runoff water.

QUATs are removed almost completely removed in WWTPs by a combination of biodegradation and sorption. However, the issue on sludge contamination remains.

Concentrations in surface water were in some cases high but very patchy. It was not possible to explain the cases with high concentrations until weather dependent sampling was initialized. High concentrations are in agreement with runoff from urban areas (roofs and terraces) during rainfall. Highest concentrations were determined after rain events, while dry weather sampling resulted in concentrations below LOQ. WWTPs can be excluded as major sources of QUATs to surface waters.

11. Perspectives

Surface waters:

During this project it could be demonstrated that QUATs occur in recipients predominately during urban rain runoff events. High concentrations in recipients are most probably linked to very local uses and wash offs with the stormwater which makes predictions considerably more difficult than prediction based on emissions from WWTPs.

Policy implications surface waters:

Measures to decrease emissions via stormwater by a) convincing the users not to use these compounds on structures like roofs and terraces will have a positive effect.

WWTPs:

The main emissions of WWTPs will be the emissions of QUATs sorbed to sludge these are brought to agricultural areas in Scandinavia, which will pose a major path of contamination.

Biodegradation does occur, thus screening for metabolites in WWTP discharges might be of relevance in the future.

Policy implications WWTPs:

a) It is currently unclear whether or not QUATs degrade on soil and if yes to which metabolites. – This would merit more research initiatives.
b) Sludge management considering sorbing organic micropollutants by disposing the sludge on agricultural land includes inherent risks as, e.g., serious loads of QUATs are introduced into the environment and the food cycle. Sludge management could be made more sustainable by i) decreasing loading of QUATs to the sludge ii) initialise a biological post treatment of sludge before amending to soils, iii) classical incineration or hydrothermal liquefaction.

Transformation products:

Both phototransformation and biodegradation (and their products) were relevant for the urban runoff samples. Biodegradation products (metabolites were relevant for wastewater treatment.

Policy implications transformation products

a) Whether or not selected transformation products are toxic will be determined by another ongoing MST project (The role of quaternary ammonium compounds and their degradation products in selection of bacterial resistance).
b) Transformation products need to be monitored and assessed more closely both in research and during the assessment and registration of products

Mechanisms controlling the emissions from urban areas.

As demonstrated in this project, most concentrations of QUATs in the recipients are due to emissions from urban areas, however very little is known concerning which mechanisms control these emissions. Most probably the concentrations on the surfaces, the depth in which the compounds are located in semi porous materials as concrete but also the wetting rate, dry-wet cycles, and thus the amount of water that reaches the respective material and the amount of water that runs down the respective material are of relevance. These will depend on rain, wind speed, wind angel, tilting angel of the respective roof or surface and flow path after the respective contacting.

Policy implications:

a) Clarifying the mechanisms of release would be beneficial.
b) Reducing use of these products on terrasses and roofs as well as other usages in the building sector linked to stormwater pipes would be beneficial for the environment.

TABLE 11.1. Overview on concentration ranges of QUATs in stormwater, wastewater and surface water in dependence to rain.

	BAC-12	BAC-14	DDAC
	[ng/L]	[ng/L]	[ng/L]
Effluent wastewater	15-35	1-5	0
Stormwater	<10-9500	<10-3500	<10-3000
Surface water (dry weather)	<1	<1	<1
Surface water (rainy weather)	6-81	<1-57	<1-14

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Appendix 1.

Appendix 1.1 Professional cleaning of the roof of a building complex. (Use of biocides in this individual operation is unknown).

Emissions of Quaternary Alkylammonium Compounds – QUAT-Fate

QUAT-fate has demonstrated that Quarternary Alkylammonium compounds (QUATs) have been and are being sold and used heavily in Denmark to “clean” roofs and terrasses. The compounds used in these products are identical to those used in biocidal applications but different to those used in fabric softeners. QUAT-fate determined that the transformation of QUATs is relevant. The respective transformation products have been characterized in the project: QUATs can be photo-oxidized which is relevant as they will be exposed to sunlight when used on rooftops and terrasses. QUATs can be metabolized by microorganisms in wastewater treatment plants, which emit these metabolites. QUATs are found at high concentrations (> 5 µg/L, i.e., exceeding the PNEC) in the discharge water from stormwater drainage systems and thus introduced into the recipients and stormwater ponds, which agrees to rumors around Denmark on fish kills due to runoff collected from roofs being directed to waters with fish populations.
QUATs were found at high concentrations (µg/L) in untreated wastewater. QUAT-Fate was able to demonstrate that removal of QUATs in wastewater treatment plants is high due to both sorption and biodegradation, thus concentration (<0.001-0.04 µg/L) in effluent wastewater that is discharged into the aquatic environment are low. In contradiction to that, the concentrations in selected recipient samples were high (0.1 µg/L). An extensive study in the recipients showed, that dry weather concentrations are low (<0.001 µg/L) while concentrations during wet weather are high (>0.1 µg/L. This behavior is consistent with the main introduction of QUATs occurring with discharged stormwater from the urban areas, while the wastewater treatment plants are a minor source.

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